SCREENING-LEVEL HAZARD CHARACTERIZATION

CHEMICAL CATEGORY NAME

Stilbene Fluorescent Brighteners

SPONSORED CHEMICALS

C.I. Fluorescent Brightener 28, free acid	**CASRN 4404-43-7**
C.I. Fluorescent Brightener 28, disodium salt	**CASRN 4193-55-9**
C.I. Fluorescent Brightener	**CASRN 13863-31-5**
C.I. Fluorescent Brightener 260, disodium salt	**CASRN 16090-02-1**
C.I. Fluorescent Brightener 220, tetrasodium salt	**CASRN 16470-24-9**
C.I. Fluorescent Brightener 235, tetrasodium salt	**CAS RN 29637-52-3**
C.I. Fluorescent Brightener 263, tetrasodium salt	**CASRN 67786-25-8**

The High Production Volume (HPV) Challenge Program[1] was conceived as a voluntary initiative aimed at developing and making publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the United States in quantities greater than one million pounds per year. In the Challenge Program, producers and importers of HPV chemicals voluntarily sponsored chemicals; sponsorship entailed the identification and initial assessment of the adequacy of existing toxicity data/information, conducting new testing if adequate data did not exist, and making both new and existing data and information available to the public. Each complete data submission contains data on 18 internationally agreed to "SIDS" (Screening Information Data Set[1,2]) endpoints that are screening-level indicators of potential hazards (toxicity) for humans or the environment.

The Environmental Protection Agency's Office of Pollution Prevention and Toxics (OPPT) is evaluating the data submitted in the HPV Challenge Program on approximately 1400 sponsored chemicals by developing hazard characterizations (HCs). These HCs consist of an evaluation of the quality and completeness of the data set provided in the Challenge Program submissions. They are not intended to be definitive statements regarding the possibility of unreasonable risk of injury to health or the environment.

The evaluation is performed according to established EPA guidance[2,3] and is based primarily on hazard data provided by sponsors; however, in preparing the hazard characterization, EPA considered its own comments and public comments on the original submission as well as the sponsor's responses to comments and revisions made to the submission. In order to determine whether any new hazard information was developed since the time of the HPV submission, a

[1] U.S. EPA. High Production Volume (HPV) Challenge Program; http://www.epa.gov/chemrtk/index.htm.
[2] U.S. EPA. HPV Challenge Program – Information Sources; http://www.epa.gov/chemrtk/pubs/general/guidocs.htm.
[3] U.S. EPA. Risk Assessment Guidelines; http://cfpub.epa.gov/ncea/raf/rafguid.cfm.

search of the following databases was made from one year prior to the date of the HPV Challenge submission to the present: (ChemID to locate available data sources including Medline/PubMed, Toxline, HSDB, IRIS, NTP, ATSDR, IARC, EXTOXNET, EPA SRS, etc.), STN/CAS online databases (Registry file for locators, ChemAbs for toxicology data, RTECS, Merck, etc.) and Science Direct. OPPT's focus on these specific sources is based on their being of high quality, highly relevant to hazard characterization, and publicly available.

OPPT may not develop HCs for those HPV chemicals which have recently been assessed and published internationally through the HPV program of the Organization for Economic Cooperation and Development (OECD) and for which Screening Initial Data Set (SIDS) Initial Assessment Reports (SIAR) and SIDS Initial Assessment Profiles (SIAP) are available. These documents are presented in an international forum that involves review and endorsement by governmental authorities around the world. OPPT is an active participant in these meetings and accepts these documents as reliable screening-level hazard assessments. HCs may be created if new data suggest a need to update the case work where the OECD document will be used as key support documentation.

These hazard characterizations are technical documents intended to inform subsequent decisions and actions by OPPT. Accordingly, the documents are not written with the goal of informing the general public. However, they do provide a vehicle for public access to a concise assessment of the raw technical data on HPV chemicals and provide information previously not readily available to the public.

Chemical Abstract Service Registry Number (CASRN)	**Sponsored Chemicals** **CASRN 4404-43-7** **CASRN 4193-55-9** **CASRN 13863-31-5** **CASRN 16090-02-1** **CASRN 16470-24-9** **CASRN 29637-52-3** **CASRN 67786-25-8**
Chemical Abstract Index Name	**Sponsored Chemicals** **Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl) amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-** **Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl) amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, disodium salt** **Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[(2-hydroxyethyl) methylamino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, disodium salt** **Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-(4-morpholinyl)-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, disodium salt** **Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, tetrasodium salt** **Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[(3-amino-3-oxopropyl) (2-hydroxyethyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, tetrasodium salt** **Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[(3-amino-3-oxopropyl) (2-hydroxyethyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, tetrasodium salt**

Structural Formula	See Appendix

Summary

The stilbene fluorescent brighteners category consists of 7 stilbene-based fluorescent whitening agents. These substances are solids possessing negligible vapor pressure and low to high water solubility. The substances contained in the stilbene fluorescent brighteners category are expected to possess low mobility in soil. Volatilization is expected to be low since these are ionic substances. Hydrolysis is expected to be negligible. The rate of atmospheric photooxidation is rapid; however, these substances are not expected to exist in the vapor phase in the ambient atmosphere. The substances in the stilbene fluorescent brightener's category are expected to possess moderate persistence (P2) and low bioaccumulation potential (B1).

Human Health Hazard

The acute oral toxicity of the stilbene fluorescent brighteners category members in rats, mice, rabbits, and dogs is low. The acute inhalation toxicity in rats is moderate (C.I. Fluorescent brightener) to high (C.I. Fluorescent brightener 28). The acute dermal toxicity in rats is low (C.I. Fluorescent brighteners 220, 235, and C.I. Fluorescent brightener) to moderate (C.I. Fluorescent brightener 263). In a combined oral chronic toxicity/carcinogenicity test, dietary exposure of rats to C.I. Fluorescent brightener 28 (free acid) for two years did not show effects considered to be treatment-related; the NOAELs were 543 and 779 mg/kg-bw/day (highest dose tested) for males and females, respectively. In a combined chronic toxicity/carcinogenicity test, dietary exposure of C.I. Fluorescent Brightener 28, disodium salt to rats for two years produced no adverse effects; the NOAEL was 500 mg/kg-bw/day (the highest dose tested). In a repeat dose study, administration of C.I. Fluorescent Brightener, via gavage, at 1000 mg/kg-bw/day for 28 days to Wistar rats resulted in hematology effects indicative of hemolytic anemia; the NOAEL was 200 mg/kg-bw/day. In a thirteen week study, repeat exposure to Fluorescent Brightener 263 by gavage to rats produced no adverse effects; the NOAEL was 300 mg/kg-bw/day (the highest dose tested). In a combined chronic toxicity/carcinogenicity test, dietary exposure of Fluorescent Brightener 220 to rats for two years produced no adverse effects; the NOAEL was 521/709 mg/kg-bw/day (the highest dose tested; males/females). In a 28 day study, repeat exposure to C. I. Fluorescent Brightener 260 by gavage to rats produced no adverse effects; the NOAEL was 1000 mg/kg-bw/day (the highest dose tested). In a two year study, dietary exposure of C. I. Fluorescent Brightener 260 to rats produced no adverse effects; the NOAEL was 524 and 791 mg/kg-bw/day (the highest dose tested) for males and females, respectively. In a two-generation reproductive toxicity study, repeated gavage exposure of albino rats to 1000 mg/kg-bw/day of C. I**.** Fluorescent Brightener resulted in increased relative and absolute kidney weights in parental females; the NOAEL (systemic toxicity; females) was 300 mg/kg-bw/day. The NOAEL (systemic toxicity; males) was 1000 mg/kg-bw/day (highest dose tested). No treatment-related effects on reproductive parameters, development, or growth of the F_1 and F_2 offspring were noted; the NOAEL (reproductive toxicity and growth and development) was 1000 mg/kg-bw/day

(highest dose tested). In a two-generation reproductive toxicity study, repeated gavage exposure of rats to C. I. Fluorescent Brightener 220 resulted in no adverse effect on reproductive performance and no adverse, test article-related changes in growth or development of offspring; the NOAEL for reproductive toxicity was 1000 mg/kg-bw/day. However, at 1000 mg/kg-bw/day there was an increase in absolute and relative kidney weights in P and F_1 dams and F_1 males. The NOAEL for systemic toxicity was 300 mg/kg-bw/day. In a prenatal developmental toxicity study, administration of C.I. Fluorescent Brightener by gavage to female Sprague-Dawley rats resulted in no adverse maternal or fetal effects; the NOAEL **(**maternal and developmental toxicity) was 1000 mg/kg-bw/day (highest dose tested). In a prenatal development study, administration of C. I. Fluorescent Brightener 220 via gavage to rabbits showed reduced discolored stools, early delivery, and abortion in the dams at 400 mg/kg-bw/day; the NOAEL for maternal toxicity and developmental toxicity was 100 mg/kg-bw/day. The stilbene fluorescent brighteners category members did not induce gene mutation or chromosomal aberrations. Excepting C.I Fluorescent brightener 260, tested members of the Stilbene fluorescent brighteners category are not irritating to rabbit skin, Stilbene fluorescent brighteners category members C.I. Fluorescent Brightener 28, free acid, Fluorescent Brightener 220, and Fluorescent Brightener 263 are not irritating to rabbit eyes. Fluorescent Brightener 28, disodium salt and Fluorescent Brightener 260 are irritating to rabbit eyes. Fluorescent Brightener 28, disodium salt, Fluorescent Brightener 260, and 263 are not skin sensitizers in guinea pigs. The stilbene fluorescent brighteners category members are not considered carcinogenic. C.I. Fluorescent Brightener 28, disodium salt and C.I. Fluorescent Brightener 220 did not induce dominant lethal mutations in mice.

Hazards to the Environment

The 96-h LC_{50} of the stilbene fluorescent brightener chemicals for fish ranges from 26 to 7,611mg/L. The 48-hour EC_{50} of the stilbene fluorescent brightener chemicals for aquatic invertebrates ranges from 6.85 to > 113 mg/L. The 72-h EC_{50} of the stilbene fluorescent brightener chemicals for aquatic plants ranges from 81 to > 100 mg/L. The 21-d LOEC and of the stilbene fluorescent brightener chemicals for aquatic invertebrates range from 2.4 to 31.6 mg/L.

No data gaps were identified under the HPV Challenge Program.

The sponsor, ETAD North America, submitted a Test Plan and Robust Summaries to EPA for stilbene fluorescent brighteners on December 21, 2005. EPA posted the submission on the ChemRTK HPV Challenge website on February 8, 2006 (http://www.epa.gov/oppt/chemrtk/pubs/summaries/stilbene/c16115tc.htm). EPA comments on the original submission were posted to the website on November 19, 2008. The sponsor submitted updated/revised documents on December 17, 2008, which were posted to the ChemRTK website on February 4, 2009. Public comments were also received and posted to the website.

Category Justification

The sponsor justified the stilbene fluorescent brighteners category based on similarities in molecular structure, environmental fate and physical chemistry properties of the seven sponsored chemicals. The same molecular structure provides the backbone for all category members. Structural variations are seen in the alkylamino substituents on the triazine and benzene rings and in the number of sulfonate groups. All category members are organic salts or internal salts and are expected to be ionized in aqueous solution. Existing aquatic and mammalian toxicity data indicate similar effects profiles for the use, release and exposure of category members. Thus, EPA agrees that the category is reasonable on the basis of structural and physical property similarities. For physical chemical properties, however, caution is needed in applying read-across between di- and tetrasulfonated substances.

Several of the sponsored substances (CASRNs 4193-55-9, 4404-43-7, 16090-02-1, 16470-24-9) have previously been assessed in the OECD HPV program and the data are available at: http://webnet.oecd.org/hpv/ui/Search.aspx. CASRN 13863-31-5 has a REACH Dossier available at: http://apps.echa.europa.eu/registered/registered-sub.aspx#search.

Justification for Supporting Chemicals

The sponsor proposed C.I. Fluorescent Brightener 28, Na^+/K^+ salt (CASRN 70942-01-7) to support the category members. However, EPA has determined that the inclusion of this compound as a supporting chemical appears not to be essential. While it is cited primarily to address the reproductive toxicity endpoint, the data provided come from other category members, principally CASRN 16470-24-9. The melting point and water solubility data simply confirm existing measured data on the two category members nearly identical to CASRN 70942-01-7.

1. Chemical Identity

1.1 Identification and Purity

When given in the robust summary, the purity of stilbene fluorescent brighteners category members varied from 17% to 100%.

1.2 Physical-Chemical Properties

The physical-chemical properties of stilbene fluorescent brighteners are summarized in Table 1. The structured are provided in the Appendix.

The stilbene fluorescent brighteners are solid substances possessing negligible vapor pressure and high water solubility.

Table 1. Physical Chemical Properties of Stilbene Fluorescent Brighteners Category[1]							
Property	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2)**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[(2-hydroxyethyl)methylamino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2)**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-(4-morpholinyl)-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2)**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, sodium salt (1:4)]**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[(3-amino-3-oxopropyl)(2-hydroxyethyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, sodium salt (1:4)**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxypropyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, sodium salt (1:4)**
CASRN	4404-43-7	4193-55-9	13863-31-5	16090-02-1	16470-24-9	29637-52-3	67786-25-8
Molecular Weight	916.98	960.95	900.90	924.92	1165.04	1219.09	1221.15
Physical State	Solid	Solid	Solid	Solid yellowish powder	Solid	Solid	Solid
Melting Point	Decomposition observed with melting at 290 °C (measured)	Decomposition observed with melting at ca. 260 °C (measured)	No data. Likely decomposes with melting above 200 °C	Decomposition observed with melting at >300 °C (measured)	Melting point above 300 °C with decomposition about 330 °C (measured)	No data. Likely decomposes with melting above 200 °C	Decomposition with an onset temperature of 350 °C (measured)
Boiling Point	Decomposes prior to boiling	Decomposes prior to boiling	Decomposes prior to boiling	Decomposes prior to boiling	Decomposes prior to boiling	Decomposes prior to boiling	Decomposes prior to boiling
Vapor Pressure	$<1.0 \times 10^{-10}$ mm Hg at 25 °C (estimated)[2]	$<1.0 \times 10^{-10}$ mm Hg at 25 °C (estimated)[2]	$<1.0 \times 10^{-10}$ mm Hg at 25 °C (estimated)[2]	$<1.0 \times 10^{-10}$ mm Hg at 25 °C (estimated)[2]	$<1.0 \times 10^{-10}$ mm Hg at 25 °C (estimated)[2]	$<1.0 \times 10^{-10}$ mm Hg at 25 °C (estimated)[2]	$<1.0 \times 10^{-10}$ mm Hg at 25 °C (estimated)[2]
Dissociation Constant (pK_a)	<2 (measured)	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Henry's Law Constant	$<1.0 \times 10^{-10}$ atm-m^3/mole (estimated)[2]	$<1.0 \times 10^{-10}$ atm-m^3/mole (estimated)[2]	$<1.0 \times 10^{-10}$ atm-m^3/mole (estimated)[2]	$<1.0 \times 10^{-10}$ atm-m^3/mole (estimated)[2]	$<1.0 \times 10^{-10}$ atm-m^3/mole (estimated)[2]	$<1.0 \times 10^{-10}$ atm-m^3/mole (estimated)[2]	$<1.0 \times 10^{-10}$ atm-m^3/mole (estimated)[2]
Water Solubility	<0.1 mg/L (estimated)[2]	ca. 50,000 mg/L at 20 °C (measured)	>1,000 mg/L (based on other category members);	1,900 mg/L at 20 °C pH 10.5 (measured); 1,800 mg/L at 20	377,000 mg/L at 20 °C (measured); 412,000 mg/L at 40 °C (measured);	>100,000 mg/L (based on other category members)	400,000 mg/L at 25 °C (measured)

			5664.9 mg/L at 25°C (estimated)[2,3]	°C pH 7 (measured);	445,000 mg/L at 60 °C (measured); 484,000 mg/L at 80 °C (measured); 512,000 mg/L at 95 °C (measured)		
Log K_{ow}	3.23 (estimated)[2]	0.65 (estimated)[2]	2.60 (estimated)[2]	-1.58 (measured)	-2.83 (estimated)[2]	-3.89 (estimated)[2]	-1.16 (estimated)[2]

[1] The ETAD Fluorescent Whitening Agent Task Force. Revised Test Plan and Robust Summary of Stilbene Fluorescent Brighteners. Available at: http://www.epa.gov/oppt/chemrtk/pubs/summaries/stilbene/c16115tc.htm as of August 10, 2011.

[2] U.S. EPA. 2011. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.10. U.S. Environmental Protection Agency, Washington, DC, USA. Available online from: http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm as of August 10, 2011.

[3] Water solubility estimates using the WSKOW program in EPIWIN yield values that do not agree with measured values. Therefore, the WATERNT v.101 program in EPIWIN was used to estimate these values; WATERNT contains a feature that allows the water solubility of one compound to be estimated from the known water solubility of a structurally similar second compound.

2. General Information on Exposure

2.1 Production Volume and Use Pattern

The Stilbene fluorescent brighteners Category chemicals had an aggregated production and/or import volume in the United States between 15 million and 100 million pounds during calendar year 2005.

- CASRN 4404-43-7: 1 million to < 10 million pounds;
- CASRN 4193-55-9: 1 million to < 10 million pounds;
- CASRN 16470-24-9: 10 million to < 50 million pounds;
- CASRN 16090-02-1: 1 million to < 10 million pounds;
- CASRN 67786-25-8: 1 million to < 10 million pounds;
- CASRN 13863-31-5: 1 million to < 10 million pounds;

CASRN 29637-52-3 was not reported in the 2006 IUR.

CASRN 4404-43-7, 67786-25-8 and 13863-31-5:
Industrial processing and uses for the chemicals were claimed confidential. No commercial and consumer uses were reported for the chemicals.

CASRN 4193-55-9:
Non-confidential information in the IUR indicated that the industrial processing and uses of the chemical include soap and cleaning compound manufacturing and textile and fabric finishing mills as other. Commercial and consumer uses for the chemical were claimed confidential.

CASRN 16470-24-9:
Non-confidential information in the IUR indicated that the industrial processing and uses of the chemical include paperboard mills, textile and fabric fishing mills, and other chemical and allied products merchant wholesalers as coloring agents, dyes; and others. Non-confidential commercial and consumer uses of this chemical include fabrics, textiles and apparel; and paper products.

CASRN 16090-02-1:
Non-confidential information in the IUR indicated that the industrial processing and uses of the chemical include soap, and cleaning compound manufacturing as other. Commercial and consumer uses for the chemical were claimed confidential.

2.2 Environmental Exposure and Fate

The environmental fate properties are provided in Table 2. The stilbene fluorescent brighteners are expected to possess low mobility in soil. Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]- (CASRN 4404-43-

7) was degraded less than 10% using a closed bottle test (OECD 301D) and was considered not readily biodegradable. Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2) (CASRN 4193-55-9) was incubated with activated domestic sludge and tested according to OECD guideline 302B (modified Zahn-Wellens test). After 24 hours, 83.6% of the material was either degraded or absorbed to the sludge. In a coupled units simulation test (OECD 303A) using aerobic activated sludge as inoculum, benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2) (CASRN 4193-55-9) was degraded 11-56% after 21 days depending upon the initial concentration of the test substance. Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-(4-morpholinyl)-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2) (CASRN 16090-02-1) was incubated with non-adapted activated sludge at 22 ± 3 °C and tested according to OECD guideline 302B. Nearly 100% degradation was observed within 28 days. Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, sodium salt (1:4)] (CASRN 16470-24-9) was not readily biodegradable achieving only 2% degradation in 28 days using the modified AFNOR (OECD 301A) test and also did not pass the Zahn-Wellens test achieving only 15% degradation within 24 hours. Volatilization of the stilbene fluorescent brighteners is low since they are ionic substances. The rate of hydrolysis is negligible for these substances. The rates of atmospheric photooxidation are expected to be rapid; however, these substances are unlikely to exist in the vapor phase in the ambient atmosphere. The stilbene fluorescent brighteners are expected to have moderate persistence (P2) and low (B1) bioaccumulation potential.

Conclusion: The stilbene fluorescent brighteners category consists of 7 stilbene-based fluorescent whitening agents. These substances are solids possessing negligible vapor pressure and low to high water solubility. The substances contained in the stilbene fluorescent brighteners category are expected to possess low mobility in soil. Volatilization is expected to be low since these are ionic substances. Hydrolysis is expected to be negligible. The rate of atmospheric photooxidation is rapid; however, these substances are not expected to exist in the vapor phase in the ambient atmosphere. The substances in the stilbene fluorescent brighteners category are expected to possess moderate persistence (P2) and low bioaccumulation potential (B1).

Table 2. Environmental Fate Properties of Stilbene Fluorescent Brighteners Category[1]							
Property	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2)**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[(2-hydroxyethyl)methylamino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2)**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-(4-morpholinyl)-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2)**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, sodium salt (1:4)]**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[(3-amino-3-oxopropyl)(2-hydroxyethyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, sodium salt (1:4)**	**SPONSORED CHEMICAL Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxypropyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, sodium salt (1:4)**
CASRN	4404-43-7	4193-55-9	13863-31-5	16090-02-1	16470-24-9	29637-52-3	67786-25-8
Photodegradation Half-life	0.5 hours (estimated)[2]	0.5 hours (estimated)[2]	0.5 hours (estimated)[2]	0.5 hours (estimated)[2]	0.6 hours (estimated)[2]	0.5 hours (estimated)[2]	0.5 hours (estimated)[2]
Hydrolysis Half-life	Stable	Stable	Stable	Stable	Stable	Stable	Stable
Biodegradation	<10% after 28 days (not readily biodegradable)	83.6% after 24 Hours (inherently biodegradable) 22-56% after 21 days (not readily biodegradable)	No data	98.8% after 28 days (inherently biodegradable)	1.2% after 28 days (not readily biodegradable) 14.8% after 24 hours (not inherently biodegradable)	No data	No data
Bioaccumulation Factor	BAF = 6.3(estimated)[2]	BAF = 6.3(estimated)[2]	BAF = 12.4 (estimated)[2]	BCF = 1.4 – 28 (measured in carp)[3]; BAF = 0.9 (estimated)[2]	BAF = 1.3 (estimated)[2]	BAF = 0.9 (estimated)[2]	BAF = 6.0 (estimated)[2]
Log K_{oc}	9.6 (estimated)[2]	9.5 (estimated)[2]	8.7 (estimated)[2]	10.3 (estimated)[2]	10.8 (estimated)[2]	9.7 (estimated)[2]	11.6 (estimated)[2]
Fugacity (Level III Model)[2]							
Air (%)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Water (%)	0.7	0.7	0.5	0.5	0.6	0.6	0.7
Soil (%)	39.1	44.4	51.0	52.1	46.5	49.3	39.4
Sediment (%)	60.2	55.0	48.4	47.4	52.9	50.2	59.9
Persistence[4]	P2 (moderate)	P2 (moderate)	P2 (moderate)	P2 (moderate)	P2 (moderate)	P2 (moderate)	P2 (moderate)
Bioaccumulation[4]	B1 (low)	B1 (low)	B1 (low)	B1 (low)	B1 (low)	B1 (low)	B1 (low)

[1] The ETAD Fluorescent Whitening Agent Task Force. Revised Test Plan and Robust Summary of Stilbene Fluorescent Brighteners. Available at: http://www.epa.gov/oppt/chemrtk/pubs/summaries/stilbene/c16115tc.htm as of August 10, 2011.

[2] U.S. EPA. 2011. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.10. U.S. Environmental Protection Agency, Washington, DC, USA. Available online from: http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm as of August 10, 2011.

[3] National Institute of Technology and Evaluation. 2002. Biodegradation and Bioaccumulation of the Existing Chemical Substances under the Chemical Substances Control Law.

Available online at http://www.safe.nite.go.jp/english/kizon/KIZON_start_hazkizon.html as of August 11, 2011.

[4] Federal Register. 1999. Category for Persistent, Bioaccumulative, and Toxic New Chemical Substances. Federal Register 64, Number 213 (November 4, 1999) pp. 60194–60204.

3. Human Health Hazard

A summary of the human health toxicity data submitted for SIDS endpoint is provided in Table 3.

Acute Oral Toxicity

C.I. Fluorescent Brightener 28, free acid (CASRN 4404-43-7)
Female Wistar II rats (10/dose) were administered C.I. Fluorescent Brightener 28, free acid, via gavage at a single 15,000 mg/kg dose and observed for 14 days following dosing. No mortalities were observed. See human health data at:
http://webnet.oecd.org/hpv/UI/Search.aspx
LD_{50} > 15,000 mg/kg

C.I. Fluorescent Brightener 28, disodium salt (CASRN 4193-55-9)
Male Wistar I rats (10/dose) were administered C.I. Fluorescent Brightener 28, disodium salt, via gavage at a single 15,000 mg/kg dose and observed for 14 days following dosing. No mortalities were observed. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
LD_{50} > 15,000 mg/kg

C.I. Fluorescent Brightener (CASRN 13863-31-5)
Albino rats (3/sex/dose) were administered C.I. Fluorescent Brightener via gavage at 5000 mg/kg and observed for 14 days following dosing, equivalent or similar to OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method). No mortalities occurred in this study.
LD_{50} > 5000 mg/kg.
http://apps.echa.europa.eu/registered/data/dossiers/DISS-9eb4a376-c9bc-1588-e044-00144f67d031/AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7_DISS-9eb4a376-c9bc-1588-e044-00144f67d031.html#AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7

C.I. Fluorescent Brightener 263, tetrasodium salt (CASRN 67786-25-8)
(1) Wistar rats (15/sex/dose) were administered C.I. Fluorescent Brightener 263, tetrasodium salt (technical grade) via an unspecified oral route at 500, 1000 or 2500 mg/kg and observed for 14 days following dosing. No mortalities were observed.
LD_{50} > 2500 mg/kg

(2) Female Wistar rats (10/sex) were administered C.I. Fluorescent Brightener 263, tetrasodium salt (solution) via an unspecified oral route at 10,000 or 15,000 mg/kg and observed for 14 days following dosing. No mortalities were observed.
LD_{50} > 15,000 mg/kg

(3) NMRI mice (15 males/group) were administered C.I. Fluorescent Brightener 263, tetrasodium salt (technical grade) via an unspecified oral route at 500 or 1000 mg/kg and observed for 14 days following dosing. No mortalities were observed.
LD_{50} > 1000 mg/kg

(4) Female New Zealand White rabbits (3/sex/group) were administered C.I. Fluorescent Brightener 263, tetrasodium salt (technical grade) via an unspecified oral route at 1000 mg/kg and observed for 14 days following dosing. No mortalities were observed.
LD_{50} > 1000 mg/kg

(5) Female Beagle dogs (2/group) were administered technical-grade C.I. Fluorescent Brightener 263, tetrasodium salt at 500 mg/kg or commercial formulation, ca. 45% C.I. Fluorescent Brightener 263, tetrasodium salt at 250 or 500 mg/kg via an unspecified oral route and observed for 14 days following dosing. No mortalities were observed.
LD_{50} > 500 mg/kg

C.I. Fluorescent Brightener 220, tetrasodium salt (CASRN 16470-24-9)
Rats (10/sex/group; strain unspecified) were administered C.I. Fluorescent Brightener 220, tetrasodium salt via gavage at 10,000 or 15,000 mg/kg and observed for 14 days following dosing. No mortalities were observed. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
LD_{50} > 15,000 mg/kg

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
Sprague-Dawley rats (5/sex) were administered C.I. Fluorescent Brightener 260, disodium salt via gavage at 5000 mg/kg and observed for 14 days following dosing. No mortalities were observed. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
LD_{50} > 5000 mg/kg

Acute Inhalation Toxicity

C.I. Fluorescent Brightener 28, free acid (CASRN 4404-43-7)
(1) Wistar II rats (10/sex/group) were exposed to C.I. Fluorescent Brightener 28, free acid, as dust at 1.82 mg/L for 1 hour and observed for 14 days following exposure. No mortalities were observed. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
1-h LC_{50} > 1.82 mg/L

(2) Wistar II rats (10/sex/group) were exposed to C.I. Fluorescent Brightener 28, free acid as dust at 0.163, 0.375, 1.225 or 1.895 mg/L for 4 hours and observed for 14 days following exposure. No mortalities were observed. See human health data at:
http://webnet.oecd.org/hpv/UI/Search.aspx
LC_{50} > 1.895 mg/L

C.I. Fluorescent Brightener (CASRN 13863-31-5)
Albino rats (5/sex/group) were exposed to C.I. Fluorescent Brightener dust via inhalation at 2.9 mg/L for 4 hours followed by a 14-day observation period. The percentage of particles in the chamber atmosphere in 2 – 5, 2 – 25 and > 25 microns in diameter range were 31%, 59%, and 10%, respectively. There were no mortalities. This IBT Laboratory study was not audited, but the results are in the range of LC_{50} values provided for other category members.
LC_{50} > 2.9 mg/L

Acute Dermal Toxicity

C.I. Fluorescent Brightener (CASRN 13863-31-5)
Wistar rats (5/sex) were exposed to C.I. Fluorescent Brightener via the dermal route at 2000 mg/kg under semi-occlusive conditions for 24 hours and observed for 15 days following dosing, according to OECD Guideline 402 (Acute Dermal Toxicity). No mortalities were observed.
LD_{50} > 2000 mg/kg
http://apps.echa.europa.eu/registered/data/dossiers/DISS-9eb4a376-c9bc-1588-e044-00144f67d031/AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7_DISS-9eb4a376-c9bc-1588-e044-00144f67d031.html#AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7

C.I. Fluorescent Brightener 263, tetrasodium salt (CASRN 67786-25-8)
Male Wistar rats (5/group) were administered technical-grade C.I. Fluorescent Brightener 263, tetrasodium salt at 500 mg/kg or commercial formulation, ca. 45% C.I. Fluorescent Brightener 263, tetrasodium salt, at 500 mg/kg via the dermal route under unspecified conditions for 7 days. No mortalities were observed.
LD_{50} > 500 mg/kg

C.I. Fluorescent Brightener 220 (CASRN 16470-24-9)
Rats (5/sex/group; strain unspecified) were administered C.I. Fluorescent Brightener 220, sodium/diethanolamine salt via the dermal route at 2000 mg/kg under unspecified conditions. The skin was washed with water 24 hours following exposure. No mortalities were observed. All animals exhibited scales at the application site while 2/5 females and 4/5 males exhibited erythema. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
LD_{50} > 2000 mg/kg

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
Wistar rats (5/sex) were exposed to C.I. Fluorescent Brightener 260, disodium salt via the dermal route at 2000 mg/kg under semi-occlusive conditions for 24 hours and observed for 15 days following dosing. No mortalities were observed. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
LD_{50} > 2000 mg/kg

Repeated-Dose Toxicity

C.I. Fluorescent Brightener 28, free acid (CASRN 4404-43-7)
In a combined chronic toxicity/carcinogenicity test, Wistar II rats (50/sex/dose) were administered C.I. Fluorescent Brightener 28, free acid (technical product; 89.1 % free acid) in the diet at 100, 1000 or 10,000 ppm (approximately 5.33/7.8, 54.08/79.97 and 542.8/779.37 mg/kg-bw/day in males/females, respectively; as calculated by the study authors) for 2 years. For all dose groups, there were no clinical signs; no increased mortality; no changes in hematological, clinical or urinary parameters; no pathological or histopathological findings and no impairment of liver or kidney function impairment. Slightly reduced body weights and statistically significant increased absolute liver weights were noted in males at 10,000 ppm. Females exhibited increased kidney and liver absolute weights (relative weights were unchanged) at the

mid- and high dose which were statistically significant at the mid-dose only. No histopathological changes were observed. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
NOAEL ~ 543/779 mg/kg-bw/day (highest dose tested; males/females)

C.I. Fluorescent Brightener 28, disodium salt (CASRN 4193-55-9)
In a combined chronic toxicity/carcinogenicity test, Sprague-Dawley rats (60/sex/dose) were administered C.I. Fluorescent Brightener 28, disodium salt in the diet at 100, 1000 or 10,000 ppm (approximately 5, 50 and 500 mg/kg-bw/day; as calculated by the study authors) for 2 years. For all dose groups, there was no increase in mortality incidence; no dose-related changes in hematological, clinical or urinary parameters, no pathological or histopathological findings and no impairment of liver or kidney function impairment. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
NOAEL ~ 500 mg/kg-bw/day (highest dose tested)

C.I. Fluorescent Brightener (CASRN 13863-31-5)
Wistar rats (5/sex/group) were administered C.I. Fluorescent Brightener, via gavage at 0, 50, 200 or 1000 mg/kg-bw/day for 28 days according to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents). No deaths were observed. Treatment-related effects (decreased erythrocyte count (by 15 to 17 %) for both sexes of high dose group, decreased hemoglobin concentration (by 8 to 23 %) for both sexes of mid and high dose groups, slightly decreased hematocrit value (by 6 to 21 %) for both sexes of mid and high dose groups, and significant differences in absolute and/or relative liver, kidney and testes weights were observed in animals of mid and high dose groups, respectively.
LOAEL=1000 mg/kg-bw/day (based on hematological changes primarily reflect a slight hemolytic anemia).
NOAEL = 200 mg/kg-bw/day
http://apps.echa.europa.eu/registered/data/dossiers/DISS-9eb4a376-c9bc-1588-e044-00144f67d031/AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7_DISS-9eb4a376-c9bc-1588-e044-00144f67d031.html#AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7

C.I. Fluorescent Brightener 263, tetrasodium salt (CASRN 67786-25-8)
Wistar rats (15/sex/dose) were administered C.I. Fluorescent Brightener 263, tetrasodium salt via gavage at 30, 100 or 300 mg/kg-bw/day for 13 weeks. No mortalities and no clinical signs were observed. There were no differences from controls in body weights, hematological parameters, clinical chemistry or urinalysis. Necropsy revealed no gross findings and no changes in organ weights.
NOAEL = 300 mg/kg-bw/day (highest dose tested)

C.I. Fluorescent Brightener 220, tetrasodium salt (CASRN 16470-24-9)
(1) In a combined chronic toxicity/carcinogenicity study, Wistar rats (50/sex/dose) were administered C.I. Fluorescent Brightener 220, tetrasodium salt in the diet at 100, 1000 or 10,000 ppm (approximately 5, 52 and 521mg/kg-bw/day in males and 7, 69 and 709 mg/kg-bw/day in females; as calculated by the study authors) for 104 weeks. No increase in mortality, no clinical signs and no change in food consumption or body weight were observed. There were no changes related to treatment in hematological, blood chemistry and urinary values noted for any group.

Males and females at 10,000 ppm exhibited a slight increase in the absolute weight of the kidneys, but there were no accompanying changes in urinary parameters and there were no gross or microscopic changes noted in the kidneys. There were also no observations of any treatment-related macroscopic or histopathological findings.
NOAEL ~ 521/709 mg/kg-bw/day (highest dose tested; males/females)

(2) Wistar rats (6/sex/dose) were administered C.I. Fluorescent Brightener 220, tetrasodium salt via gavage at 30, 60, 120, 250 or 500 mg/kg-bw/day for 5 days/week for 10 weeks. No mortalities and no treatment-related changes in clinical signs, body weights, blood and urine parameters, gross examination or organ weights were noted.
NOAEL = 500 mg/kg-bw/day (highest dose tested)

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
(1) Wistar rats (5/sex/group) were administered C.I. Fluorescent Brightener 260, disodium salt (FWA-1) via gavage at 0, 50, 200 or 1000 mg/kg-bw/day for 28 days followed by a 14-day observation period. No mortality and no treatment-related changes in clinical signs, body weights, blood and urine parameters, gross examination or relative or absolute organ weights when compared to controls were noted. No changes were noted upon gross and macroscopic examination of male and female reproductive organs and no effects were observed on absolute and relative weights of these organs. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
NOAEL = 1000 mg/kg-bw/day (highest dose tested)

(2) Wistar rats (50/sex/group) were administered C.I. Fluorescent Brightener 260, disodium salt (FWA-1) via the diet at 0, 100, 1000 or 10,000 ppm (approximately 0, 4.9, 51.4 and 523.9 mg/kg-bw/day in males and 0, 7.5, 77.5 and 790.6 mg/kg-bw/day in females) for 24 months. No mortalities and no treatment-related clinical signs were observed. Body weight gain and food consumption were comparable to control animals. Increased organ weights at 10,000 ppm, including absolute kidney and liver weights in males and absolute ovary weight in females were not accompanied by hematological, biochemical or histopathological changes. Assessment of hematological, clinical chemistry and urinalysis data revealed no treatment-related effects. No changes were noted upon gross and macroscopic examination of male and female reproductive organs and no effects were observed on absolute and relative weights of these organs.
NOAEL ~ 524/791 mg/kg-bw/day (highest dose tested; males/females)

Reproductive Toxicity

C.I. Fluorescent Brightener (CASRN 13863-31-5)
In a two-generation reproductive toxicity study, CD (Crl:CD (SD)IGS BR) albino rats (26/sex/group) were administered C.I. Fluorescent Brightener at 0, 100, 300 or 1000 mg/kg-bw/day via gavage according to EPA OPPTS 870.3800 (Reproduction and Fertility Effects). Treatment of the parental (P) generation began 10 weeks prior to mating and continued until euthanasia. F_1 and F_2 generation offspring were potentially exposed in utero and during lactation. The F_1 offspring selected to comprise the F_1 parental group were exposed for ≥70 days prior to mating (beginning at age ≥28 days), until euthanasia. In parental rats, the only treatment-related effect was an increase in absolute and relative kidney weights in the females. No treatment-

related effects of the parental males were noted at 1000 mg/kg-bw/day. No treatment-related effects on reproductive parameters in P or F_1 parents were observed. No adverse, treatment-related changes were observed in the development or growth of the F_1 and F_2 offspring.

LOAEL parental female (systemic toxicity) = 1000 mg/kg-bw/day (based on increased relative and absolute kidney weight)
NOAEL parental female (systemic toxicity) = 300 mg/kg-bw/day
NOAEL parental male (systemic toxicity) = 1000 mg/kg-bw/day (highest dose tested)
NOAEL F_1 male and female (toxicity) = 1000 mg/kg-bw/day (highest dose tested)
NOAEL P and F_1 (reproductive toxicity) = 1000 mg/kg-bw/day (highest dose tested)
NOAEL F_1 and F_2 (growth and development) = 1000 mg/kg-bw/day (highest dose tested)
http://apps.echa.europa.eu/registered/data/dossiers/DISS-9eb4a376-c9bc-1588-e044-00144f67d031/AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7_DISS-9eb4a376-c9bc-1588-e044-00144f67d031.html#AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7

C.I. Fluorescent Brightener 220, tetrasodium salt (CASRN 16470-24-9)
(1) In a two-generation reproductive toxicity study, Sprague-Dawley rats (26/sex/group) were administered C.I. Fluorescent Brightener 220, tetrasodium salt at 0, 100, 300 or 1000 mg/kg-bw/day via gavage for ~ 36 weeks (10 weeks pre-mating, mating, gestation and lactation until euthanasia) . The F_1 offspring selected to continue as F_1 parents received C.I. Fluorescent Brightener 220 via gavage for at least 70 days prior to mating until euthanasia. In parental rats (P and F_1 parents), the only treatment-related effect was an increase in absolute and relative kidney weights in P and F_1 dams and F_1 males at 1000 mg/kg-bw/day. No treatment-related effects on reproductive parameters in P or F_1 parents were observed. No adverse, treatment-related changes were observed in the development or growth of the F_1 and F_2 offspring. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
LOAEL (systemic toxicity) = 1000 mg/kg-bw/day (based on increased relative and absolute kidney weight)
NOAEL (systemic toxicity) = 300 mg/kg-bw/day
NOAEL (reproductive toxicity) = 1000 mg/kg-bw/day (highest dose tested)

(2) In a range-finding study for the two-generation study described above, Sprague-Dawley rats (10/sex/dose) were administered C.I. Fluorescent Brightener 220, tetrasodium salt via gavage at 30, 100, 300 or 1000 mg/kg-bw/day from 28 days pre-mating to day 4 of lactation in females and pups. Males were sacrificed after mating. No treatment-related clinical signs were noted in adults from pre-mating to scheduled sacrifice. No changes in body weight or food consumption were noted. No changes were noted at the necropsy of the adult rats. Fertility was unaffected. Mating, fertility and fecundity indices were comparable to controls. The numbers of females delivering litters with liveborn or stillborn pups, gestation length and gestation index were unaffected by treatment. The number of pups/litter and number surviving to day 4 were comparable to controls. Pup body weight and gross appearance were unaffected by treatment.

Developmental Toxicity

C.I. Fluorescent Brightener (CASRN 13863-31-5)
Female Sprague-Dawley rats (30/ females/dose) were administered C.I. Fluorescent Brightener by gavage at 100, 400 or 1000 mg/kg-bw/day on gestational days 6 – 19 according to EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study). No adverse maternal or fetal effects were observed following the administration of 100, 400, or 1000 mg/kg/day to rats between gestational days 6 and 19.
NOAEL (maternal and developmental toxicity) = 1000 mg/kg-bw/day (highest dose tested).
http://apps.echa.europa.eu/registered/data/dossiers/DISS-9eb4a376-c9bc-1588-e044-00144f67d031/AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7_DISS-9eb4a376-c9bc-1588-e044-00144f67d031.html#AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7

C.I. Fluorescent Brightener 220, tetrasodium salt (CASRN 16470-24-9)
(1) Female New Zealand White rabbits (25/group) were administered C.I. Fluorescent Brightener 220, tetrasodium salt at 100, 400 or 800 mg/kg-bw/day via gavage on gestational days 7 – 28. Mortality due to gavage error occurred in the control, low-dose and mid-dose groups (2/25, 1/25 and 1/25 respectively). The group receiving 800 mg/kg-bw/day was terminated prior to the completion of the study due to mortality (8/25 found dead; 1/25 euthanized) or morbidity. Abortion occurred in 7/25 and clinical signs in those animals included convulsions, decreased defecation, soft stool, discolored feces and reddish fluid in the refuse pan along with decreases in body weight gain and food consumption. Necropsy revealed edematous stomachs, red discolored and/or edematous intestines, bloody and/or mucoid intestine contents and foci in the lungs. At 400 mg/kg-bw/day, aside from one mortality due to gavage error, there were no treatment-related mortalities and no changes in body weight, body weight gain or food consumption. Slight increases in reduced, soft or discolored stools were noted. Treatment-related effects at 400 mg/kg-bw/day included findings of an edematous stomach and liquid and bloody contents in the intestines of one doe that aborted and two does that delivered early. No treatment-related effects were noted at 100 mg/kg-bw/day. At 100 and 400 mg/kg-bw/day, there were no observed effects on the number of corpora lutea, implantation, live fetuses, resorptions, uterine weights and adjusted body weight gain. Developmental effects such as decreased fetal body weights, increased incidence of hemorrhagic iris, gallbladder hypogenesis, hypoplasia of the gallbladder and azygous lobe of the lung absent were considered to be spontaneous as the frequency did not exceed historical incidence. External and skeletal examinations also did not reveal treatment-related effects. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
LOAEL (maternal toxicity) = 400 mg/kg-bw/day (based on reduced, soft or discolored stools, early delivery and abortion)
NOAEL (maternal toxicity) = 100 mg/kg-bw/day
LOAEL (developmental toxicity) = 400 mg/kg-bw/day (based on fetal toxicity)
NOAEL (developmental toxicity) = 100 mg/kg-bw/day

(2) Female New Zealand White rabbits (7/group) were administered C.I. Fluorescent Brightener 220, tetrasodium salt at 30, 300 or 1000 mg/kg-bw/day via gavage on gestational days 7 – 28. Excessive maternal toxicity occurred at 1000 mg/kg-bw/day as exhibited by death, abortion, increased incidence of clinical and gross pathological findings and marked decreases in body

weight and food consumption. None of the animals in the 1000 mg/kg-bw/day group survived to study completion. At 30 and 300 mg/kg-bw/day, no adverse maternal systemic toxicity or effects on the number of corpora lutea, implantations, live fetuses, pre- or post-implantation loss or resorptions were observed. The fetuses were not examined in this pilot study. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
LOAEL (maternal and developmental toxicity) = 1000 mg/kg-bw/day (based on mortality, abortions, increased incidence of gross and pathological findings and decreased body weights and food consumption)
NOAEL (maternal and developmental toxicity) = 300 mg/kg-bw/day

(3) Female Sprague-Dawley rats (30/dose) were administered C.I. Fluorescent Brightener 220, tetrasodium salt by gavage at 100, 400 or 1000 mg/kg-bw/day on gestational days 6 – 19. Discolored feces was noted in dams from the 400 and 1000 mg/kg-bw/dose groups. Necropsy revealed no gross changes in the dams and no effects on body weight, body weight gain, food consumption, number of corpora lutea, implantations, live fetuses, preimplantation, postimplantation or resorption rates were observed at any dose level. Skeletal examination of the fetuses revealed slight increases in the number of vertebral malformations at 1000 mg/kg-bw/day and of rudimentary ribs at 100 and 1000 mg/kg-bw/day and the incidence of misaligned sternebra in all dose groups. Since these changes were either within the range of incidence in historical controls or had no dose-relationship, they were not considered effects of treatment. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
NOAEL (maternal and developmental toxicity) = 1000 mg/kg-bw/day (highest dose tested)

(4) Female Sprague-Dawley rats (10/dose) were administered C.I. Fluorescent Brightener 220, tetrasodium salt by gavage at 30, 300 or 1000 mg/kg-bw/day on gestational days 6 – 19. No treatment-related clinical signs were observed at any dose, necropsy reveals no gross changes in the dams and no effects on body weight, body weight gain, food consumption, number of corpora lutea, implantations, live fetuses, preimplantation, postimplantation or resorption rates were observed at any dose level. No adverse maternal or developmental effects were observed during this study. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
NOAEL (maternal and developmental toxicity) = 1000 mg/kg-bw/day (highest dose tested)

Genetic Toxicity – Gene Mutation

In vitro
C.I. Fluorescent Brightener 28, disodium salt (CASRN 4193-55-9)
(1) *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to C.I. Fluorescent Brightener 28, disodium salt, at 0, 8, 40, 200, 1000 or 5000 µg/plate with and without S9 metabolic activation. No cytotoxicity was noted in strain TA100 at the highest dose tested. Positive and solvent control tests were conducted, giving the expected results. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, disodium salt was not mutagenic in this assay.

(2) *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* strain WP2uvrA were exposed to C.I. Fluorescent Brightener 28, disodium salt

at 0, 33, 100, 333, 2500 or 5000 µg/plate with and without S9 metabolic activation. Appropriate negative and positive controls were tested concurrently and gave the expected results. No cytotoxicity was noted. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, disodium salt was not mutagenic in this assay.

C.I. Fluorescent Brightener (CASRN 13863-31-5)
Salmonella typhimurium strains A 1535, TA 1537, TA 98 and TA100and *Escherichia coli* strain WP2uvrA were exposed to C.I. Fluorescent Brightener at 0, 33, 100, 333, 2500 or 5000 µg/plate with and without S9 metabolic activation according to OECD Guideline 471 (Bacterial Reverse Mutation Assay). Negative and positive controls were tested concurrently and gave the expected results. No cytotoxicity was noted.
http://apps.echa.europa.eu/registered/data/dossiers/DISS-9eb4a376-c9bc-1588-e044-00144f67d031/AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7_DISS-9eb4a376-c9bc-1588-e044-00144f67d031.html#AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7
C.I. Fluorescent Brightener was not mutagenic in this assay

C.I. Fluorescent Brightener 220 (CASRN 16470-24-9)
(1) *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 were exposed to C.I. Fluorescent Brightener 220 at concentrations up to 2500 µg/plate with and without S9 metabolic activation. Positive and negative controls were tested concurrently. No cytotoxicity was observed up to the highest dose tested.
C.I. Fluorescent Brightener 220 was not mutagenic in this assay.

(2) *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1538 and TA1537 were exposed to C.I. Fluorescent Brightener 220 at 10, 100, 333.3, 1000 or 5000 µg/plate with and without S9 metabolic activation. Positive and negative controls were tested concurrently. A slight precipitate was noted at 5000 µg/plate; however, a homogenous suspension was obtained and the precipitate had no influence on the data recorded. No cytotoxicity was observed up to the highest dose tested.
C.I. Fluorescent Brightener 220 was not mutagenic in this assay.

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
Salmonella typhimurium strains TA98, TA100, TA1535, TA1538 and TA1537 were exposed to C.I. Fluorescent Brightener 260, disodium salt (FWA-1) at 10.0, 100, 333.3, 1000 or 5000 µg/plate with and without S9 metabolic activation. Appropriate positive and negative controls were tested concurrently and the results were as expected. Cytotoxicity was observed only in strain TA98 at 5000 µg/plate without S9 metabolic activation. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt was not mutagenic in this assay.

Genetic Toxicity – Chromosomal Aberrations

In vitro

C.I. Fluorescent Brightener (CASRN 13863-31-5)
Chinese hamster lung fibroblasts (V79) were exposed to C.I. Fluorescent Brightener at concentrations ranging from 0.1 to 5 mg/ml with and without activation according to OECD Guideline 473 (*In vitro* Mammalian Chromosome Aberration Test). The test substance did not induce Chromosome Aberrations in this test.
C.I. Fluorescent Brightener was negative for chromosomal aberrations in this assay.

C.I. Fluorescent Brightener 220 (CASRN 16470-24-9)
Chinese hamster V79 cells were exposed to C.I. Fluorescent Brightener 220 at concentrations ranging from 0.3 to 5.0 mg/L with and without metabolic activation. Negative and appropriate controls were tested concurrently. A single observation of an increased aberration rate with metabolic activation was observed at the fixation interval of 7 hours at 5 mg/L. However, only one slide could be scored and an independent second experiment could not confirm this result. Therefore, it was considered that the test substance did not induce structural chromosome aberrations. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
C.I. Fluorescent Brightener 220 was negative for chromosomal aberrations in this assay.

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
(1) Chinese hamster V79 cells were exposed to C.I. Fluorescent Brightener 260, disodium salt at 10, 100 or 150 µg/mL with and without S9 activation. Negative and appropriate positive controls were tested concurrently. Preparation of chromosomes took place at 7 hours (high dose), 18 hours (low, medium and high doses) and 28 hours (high dose) following an incubation of 4 hours. There was evidence of cytotoxicity at 150 µg/mL with and without S9 mix. An increase in chromosomal aberrations was observed in the positive controls. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt did not induce chromosomal aberrations in this assay.

(2) Chinese hamster lung (CHL) fibroblast cells were exposed to C.I. Fluorescent Brightener 260, disodium salt at 0.03 mg/mL for 24 or 48 hours of incubation. Solvent controls were tested concurrently. One hundred well-spread metaphases were examined for chromatid gaps, chromatid breaks, chromatid or chromosomal translocation, ring formation and fragmentation or pulverization. The incidence of polyploidy cells was also calculated. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt did not induce chromosomal aberrations in this assay.

(3) Chinese hamster ovary (CHO) cells were exposed to C.I. Fluorescent Brightener 260, disodium salt at 0.1, 0.01 or 0.001 mM dissolved in dimethylsulfoxide (DMSO) in a sister chromatid exchange assay. No evidence of mitosis was observed at 0.1 mM and the mitotic index was decreased more than 50% of control at 0.01 mM. No evidence of toxicity was

observed at 0.001 mM. The incidence of sister chromatid exchanges and chromosome aberrations at 0.01 and 0.001 mM was comparable to controls. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt did not induce sister chromatid exchanges in this assay.

In vivo

C.I. Fluorescent Brightener (CASRN 13863-31-5)
(1) Chinese hamsters (3/sex/group) were administered C.I. Fluorescent Brightener via gavage at 1250, 2500 or 5000 mg/kg-bw/day for 2 consecutive days. Positive and negative controls were also tested. Bone marrow was harvested 24 hours after the final dose. One thousand cells per animal were examined for nuclear anomalies, including single Jolly bodies, nucleus fragments in erythrocytes, micronuclei and necrobiotic cells.
C.I. Fluorescent Brightener did not induce chromosomal aberrations in this assay.
http://apps.echa.europa.eu/registered/data/dossiers/DISS-9eb4a376-c9bc-1588-e044-00144f67d031/AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7_DISS-9eb4a376-c9bc-1588-e044-00144f67d031.html#AGGR-21ee66f5-a0ce-46f3-88a9-0cb0952e4be7

(2) Chinese hamsters (2/sex/group) were administered C.I. Fluorescent Brightener via gavage at 1250, 2500 or 5000 mg/kg-bw/day for 2 consecutive days. Positive and negative controls were also tested. The hamsters were injected i.p. with colcemid 2 hours after the final dose and bone marrow was harvested 4 hours following the administration of colcemid. One hundred metaphase plates per animal were examined for chromatid- and chromosome-type aberrations, gaps and pulverizations.
C.I. Fluorescent Brightener did not induce chromosomal aberrations in this assay.

C.I. Fluorescent Brightener 220, tetrasodium salt (CASRN 16470-24-9)
(1) In a cytogenetics assay, male Chinese hamsters (8/group) were administered two doses of C.I. Fluorescent Brightener 220 by gavage at 5000 mg/kg-bw within 24 hours. Forty-eight hours following the last treatment, 100 metaphases per animal were prepared from spermatogonia. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
C.I. Fluorescent Brightener 220, tetrasodium salt did not induce chromosomal aberrations in this assay.

(2) NMRI mice (5/sex/group) were administered C.I. Fluorescent Brightener 220 in two doses via gavage within 24 hours at 4735 mg/kg-bw (commercial formulation) or 5000 mg/kg-bw (technical product). Positive and vehicle controls were tested concurrently. Study details were not provided. There were no signs of toxicity and no effect on the number of micronuclei and PCE/MCE ratio compared to controls. The incidence of micronuclei was greatly increased in the positive controls. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
C.I. Fluorescent Brightener 220, tetrasodium salt did not increase the number of micronuclei or change the ratio of PCE/MCE in this assay.

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)

(1) NMRI mice (5/sex) were administered C.I. Fluorescent Brightener 260, disodium salt via gavage at 5000 mg/kg-bw and bone marrow was collected at 24, 48 and 72 hours and prepared for analysis of the induction of micronuclei in PCEs. Negative and positive controls were tested and expected results were obtained. Slight cytotoxicity was noted at the administered concentration the number of normo-chromatic erythrocytes was increased compared to controls after dosing. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt did not increase the number of micronuclei in this assay.

(2) Chinese hamsters (2/sex/group) were administered C.I. Fluorescent Brightener 260, disodium salt via gavage at 1250, 2500 or 5000 mg/kg-bw/day for 2 consecutive days. Bone marrow was harvested from the femurs prepared for metaphase analysis 4 hours following the administration of colcemid. Appropriate positive and negative controls were tested and returned expected results. Treated animals showed no difference in the incidence of chromatid-type aberrations, chromosomal aberrations, chromosome gaps or pulverizations from negative controls. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt did not induce chromosomal aberrations in this assay.

(3) Chinese hamsters (3/sex/group) were administered C.I. Fluorescent Brightener 260, disodium salt via gavage at 1250, 2500 or 5000 mg/kg-bw/day for 2 consecutive days. Positive and negative controls were also tested. Bone marrow was harvested 24 hours after the final dose. One thousand cells per animal were examined for nuclear anomalies, including single Jolly bodies, nucleus fragments in erythrocytes, micronuclei in erythroblasts and leucopoietic cells, bizarre forms of nuclei and necrobiotic cells. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener did not induce chromosomal aberrations in this assay.

Additional Information

Skin Irritation

C.I. Fluorescent Brightener 28, free acid (CASRN 4404-43-7)

Two New Zealand White rabbits were exposed to 500 mg of C.I. Fluorescent Brightener 28, free acid as a dry powder applied to the inner surface the ears and covered with adhesive bandages for 24 hours. The ears were rinsed following exposure and the rabbits were observed for 7 days. No signs of skin irritation were observed. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, free acid was not irritating to rabbit skin in this study.

C.I. Fluorescent Brightener 28, disodium salt (CASRN 4193-55-9)

(1) Rabbits (6/group; strain and sex not specified) were administered C.I. Fluorescent Brightener 28, disodium salt in water at 50% (by weight or volume not specified) on intact or scarified skin and covered with occlusive bandages for 24 hours. Erythema and edema were not observed

during the 14-day observation following exposure. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, disodium salt was not irritating to rabbit skin in this study.

(2) New Zealand White rabbits (3/sex) were administered 500 mg of moistened C.I. Fluorescent Brightener 28, disodium salt on shaved intact and scarified skin (two sites/rabbit) and covered with occlusive bandages for 24 hours. No signs of irritation or clinical signs were observed during the 14-day observation period following exposure. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, disodium salt was not irritating to rabbit skin in this study.

C.I. Fluorescent Brightener 263, tetrasodium salt (CASRN 67786-25-8)
In three dermal irritation studies, rabbits (sex and strain unspecified) were administered unspecified quantities of one of three formulations of C.I. Fluorescent Brightener 263, tetrasodium salt: a liquid formulation of sodium/potassium salt, technical grade, ca. 28% water free acid; technical grade (100% purity); or a commercial formulation, ca. 45% C.I. Fluorescent Brightener 263, tetrasodium salt. The test materials were applied to the inner ear under semi-occluded conditions for 24 hours. All formulations were not irritating to rabbit skin.
C.I. Fluorescent Brightener 263, tetrasodium salt was not irritating to rabbit skin in this study.

C.I. Fluorescent Brightener 220 (CASRN 16470-24-9)
Rabbits (2/group; strain unspecified) were administered C.I. Fluorescent Brightener 220, disodium salt (purity 85.5%) or C.I. Fluorescent Brightener 220 (purity unspecified) to the ear in two dermal irritation studies. Irritation scoring was according to Draize. No additional details were provided. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
C.I. Fluorescent Brightener 220 was not irritating to rabbit skin in this study.

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
(1) Rabbits were exposed to C.I. Fluorescent Brightener 260 at 50% concentration in a vehicle of propylene glycol and saline via the dermal route under occlusive conditions for 24 hours. Additional study details were not given. The results were noted as moderately irritating. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt was moderately irritating to rabbit skin in this study.

(2) New Zealand White rabbits (3/sex) were administered C.I. Fluorescent Brightener 260, disodium salt via application of saturated gauze to the shaved, abraded or intact skin under occlusive conditions for 24 hours. Skin reactions were assessed at 24, 48 and 72 hours and 4 and 7 days. The primary dermal irritation index (PDII) was 2.33. The results were noted as moderately irritating. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt was moderately irritating to rabbit skin in this study.

Eye Irritation

C.I. Fluorescent Brightener 28, free acid (CASRN 4404-43-7)
Two New Zealand White rabbits were administered dry C.I. Fluorescent Brightener 28, free acid by the instillation of 50 mg into one conjunctival sac followed by a 7-day observation period. The eyes were examined at 1 and 24 hours, then daily. No reactions were observed in any treated eyes. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, free acid was not irritating to rabbit eyes in this study.

C.I. Fluorescent Brightener 28, disodium salt (CASRN 4193-55-9)
(1) New Zealand White rabbits (3/sex) were administered C.I. Fluorescent Brightener 28, disodium salt by the instillation of 100 mg into one eye. The eyelids were held closed for 1 second. Examination of the eyes was aided with fluorescein dye 24 hours following instillation. Redness of the conjunctiva was observed at 24 hours in all rabbits. At 72 hours, no evidence of irritation was observed in any treated eyes. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, disodium salt was slightly irritating to rabbit eyes in this study.

(2) New Zealand White rabbits (3/sex) were administered C.I. Fluorescent Brightener 28, disodium salt by the instillation of 100 mg into one eye. The eyelids were held closed for 1 second. The eyes were examined 24, 48 and 72 hours and 7 days following application. Examination at 24 hours was aided with fluorescein dye. No reactions were observed in any treated eyes.
C.I. Fluorescent Brightener 28, disodium salt was not irritating to rabbit eyes in this study.

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
In two studies, New Zealand White rabbits (3/sex) were administered 100 mg C.I. Fluorescent Brightener 260, disodium salt at 62 or 82% active ingredient via instillation into the conjunctival sac of the left eye, then the lids were held closed for a few seconds. Approximately 30 seconds following treatment, 3/6 treated eyes were rinsed with water. Rinsed and unrinsed eyes were scored independently. The primary irritation index for the rinsed eyes was 1.2 and for unrinsed eyes, 12.1. The results were noted as slightly irritating and the classification as not irritating. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt was slightly irritating to rabbit eyes in this study.

C.I. Fluorescent Brightener 220 (CASRN 16470-24-9)
Rabbits (2/group; strain unspecified) were administered C.I. Fluorescent Brightener 220, disodium salt (purity 85.5%) or C.I. Fluorescent Brightener 220 (purity unspecified) to the eye in two eye irritation studies. The eyes were scored according to Draize. No additional details were provided. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
C.I. Fluorescent Brightener 220 was not irritating to rabbit eyes in this study.

C.I. Fluorescent Brightener 263, tetrasodium salt (CASRN 67786-25-8)
Rabbits (sex and strain unspecified) were administered 50 mg of one of three formulations of C.I. Fluorescent Brightener 263, tetrasodium salt: a liquid formulation of sodium/potassium salt, technical grade, ca. 28% water free acid; technical grade (100% purity); or a commercial formulation, ca. 45% C.I. Fluorescent Brightener 263, tetrasodium salt, sodium salt. The test materials were applied to the eye in an unspecified manner. Only C.I. Fluorescent Brightener 263, tetrasodium salt, liquid formulation of sodium/potassium salt, technical grade ca. 28% water free acid induced slight reddening of the conjunctivae.
C.I. Fluorescent Brightener 263, tetrasodium salt was not irritating to rabbit eyes in this study.

Sensitization

C.I. Fluorescent Brightener 28, disodium salt (CASRN 4193-55-9)
C.I. Fluorescent Brightener 28, disodium salt was tested for sensitization potential in a guinea pig maximization test. Dunkin-Hartley guinea pigs (10/sex/treatment group) were induced first by intradermal injection (i.d.) with 1% C.I. Fluorescent Brightener 28, disodium salt in distilled water, Freund's complete adjuvant or both. One week following the initial induction, a second induction with 15% C.I. Fluorescent Brightener 28, disodium salt in petrolatum was applied to the epidermis with occlusive covering for 48 hours. The first challenge was conducted 2 weeks after the epidermal induction, followed in 2 weeks by a second challenge. Concurrent testing of a positive and vehicle control were conducted. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, disodium salt was not sensitizing to guinea pigs in this study.

C.I. Fluorescent Brightener 263, tetrasodium salt (CASRN 67786-25-8)
(1) The sensitization potential of C.I. Fluorescent Brightener 263, tetrasodium salt was tested in guinea pigs (five males, nine females; strain unspecified) via an initial intracutaneous injection of 0.05 mL of a 0.1% suspension of C.I. Fluorescent Brightener 263, tetrasodium salt in physiological saline followed by nine additional induction injections of 0.1 mL of the suspension. The challenge, 0.5 mL of the suspension, was administered 14 days after the final induction injection via an unspecified method. No sensitization reactions were noted.
C.I. Fluorescent Brightener 263, tetrasodium salt was not sensitizing to guinea pigs in this study.

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
Female Ibm:GOHI (SPF) guinea pigs were tested for sensitization potential by C.I. Fluorescent Brightener 260, disodium salt in a guinea pig maximization test. The induction phase included intradermal injection of 1% C.I. Fluorescent Brightener 260, disodium salt in saline or diluted to 1% in a mixture of Freund's adjuvant:physiological saline (50:50) followed 7 days later by epidermal application of 25% C.I. Fluorescent Brightener 260, disodium salt in vaselinum album under occluded conditions for 24 hours. The challenge phase included epidermal application of 25% C.I. Fluorescent Brightener 260, disodium salt in vaselinum album under occluded

conditions for 18 hours. No evidence of sensitization was noted. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt was not sensitizing to guinea pigs in this study.

Carcinogenicity

C.I. Fluorescent Brightener 28, free acid (CASRN 4404-43-7)
(1) In a 2-year dietary combined chronic toxicity/carcinogenicity study on Wistar rats (50/sex/group) described previously, administration of C.I. Fluorescent Brightener 28, disodium salt at 100, 1000 or 10,000 ppm (~ 5.33/7.8, 54.08/79.97 and 542.8/779.37 mg/kg-bw/day in males/females, respectively; as calculated by the study authors) had no effect on tumor incidence. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, free acid was not carcinogenic in this study.

(2) In a study designed to detect the effects of substances that alter the ultraviolet (UV) radiation mediated cutaneous carcinogenesis, albino-hairless mice (50/sex) were exposed to a 0.01% solution (100 mg/L) of C.I. Fluorescent Brightener 28, via dermal application to the back 1 hour prior to UV exposure. An untreated control group, a vehicle control group (0.005% Emulgator K30) and a control group treated with acetone were tested concurrently. All groups received daily UV-irradiation for 4 hours/day for 320 days. C.I. Fluorescent Brightener 28, disodium salt had no influence on the time of tumor formation, the number of animals with tumors, total number of tumors and tumor growth as compared to controls. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, free acid was not carcinogenic in this study.

C.I. Fluorescent Brightener 28, disodium salt (CASRN 4193-55-9)
(1) In a 2-year dietary combined chronic toxicity/carcinogenicity study in Sprague-Dawley rats (60/sex/group) described previously, the development of mammary gland adenocarcinomas was noted in all female groups unrelated to dose with the incidence increasing with age. Pituitary adenomas and cystic adrenal glands were also observed in females. These and other tumors observed were not considered to be treatment-related. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, disodium salt was not carcinogenic in this study.

(2) Mice (outbred albino CD-1; 75/sex/group) were administered C.I. Fluorescent Brightener 28, disodium salt at 0, 0 (two control groups), 100, 1000 or 10,000 ppm (~ 0, 0, 17.2, 172 and 1722 mg/kg-bw/day) in the diet for 18 months. Findings included adenosarcomas of the liver and mammary glands, sarcomas in various tissues and leukemias in control mice. The neoplasms were distributed randomly among treated and control groups. The study authors concluded that C.I. Fluorescent Brightener 28, disodium salt was not carcinogenic to mice under the conditions of this study. This is a TSCATS study (EPA Doc. No. 88-90007008; OTS0545383). See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, disodium salt was not carcinogenic in this study.

(3) Groups of 96 male C3H mice were exposed to UV light 3 times weekly immediately following the skin application of C.I. Fluorescent Brightener 28, disodium salt in solution with a wetting agent (detergent matrix) at 7.9 or 0.98%, detergent only (vehicle control) or positive control (0.01% solution of 8-methoxypsoralen in acetone) for 320 days. All mice exposed to UV light developed skin tumors; however, those exposed to the optical brightener had fewer tumors, with fewer animals developing tumors and a longer latency time to tumor development when compared with the vehicle control. See human health data at: http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, disodium salt was not carcinogenic in this study.

C.I. Fluorescent Brightener 220 (CASRN 16470-24-9)
(1) In the 104-week dietary combined chronic toxicity/carcinogenicity study on Wistar rats (50/sex/group) described previously, no indication of carcinogenic effects was noted. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
C.I. Fluorescent Brightener 220 was not carcinogenic in this study.

(2) In a study on the photocarcinogenicity, male and female albino-hairless mice (50/sex/treatment group) were administered 0.03 mL of a 0.01% of C.I. Fluorescent Brightener 220, disodium salt via the dermal route 3 times/week for 320 days in addition to being exposed to ultraviolet radiation for 4 hours/days, 7 days/week. Three control groups (radiation only, acetone only and vehicle only) were included in the study. There was no effect on mortality and no influence on time of tumor formation, number of animals with tumors and growth of tumors. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
C.I. Fluorescent Brightener 220 was not carcinogenic in this study.

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
(1) In the 2-year combined oral chronic toxicity/carcinogenicity study in Wistar rats described previously, although a number of benign and malignant neoplasms were found, statistical analysis revealed no differences from controls and the tumor incidences were not organ or neoplastic class specific. Therefore, the findings were not regarded as biologically significant. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt was not carcinogenic in this study.

(2) Male and female hairless mice, strain SKH/HR1were dermally treated with 30 µL of a 0.001 or 0.01% solution of C.I. Fluorescent Brightener 260, disodium salt via the dermal route under unspecified conditions 3 times/week for 700 days. Three negative control groups and one positive control group (0.01% solution of 8-methoxypsoralen in acetone) were included in the study. See human data at: http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt was not carcinogenic in this study.

(3) Male and female hairless mice, strain SKH/HR1were dermally treated with 30 µL of a 0.001 or 0.01% solution of C.I. Fluorescent Brightener 260, disodium salt via the dermal route under unspecified conditions 3 times/week for 265 days followed by a 100-day observation period. Three negative control groups and one positive control group (0.01% solution of 8-

methoxypsoralen in acetone) were included in the study. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt was not carcinogenic in this study.

(4) Groups of 96 male C3H mice were exposed to UV light at 1.0E6 erg/cm^2 3 times weekly immediately following the skin application of C.I. Fluorescent Brightener 260, disodium salt at 0, 0.01, 0.1 or 0.15 mg/kg-bw/day in methanol/water or detergent/water. Control groups were administered methanol/water, detergent/water or nothing, followed by UV exposure of comparable duration and exposure as the treated groups. Findings include squamous and basal cell carcinomas, keratoacanthomas, fibromas, sarcomas and other lesions typical of UV-irradiated skin occurring randomly in all UV-exposed groups. The authors concluded that C.I. Fluorescent Brightener 260, disodium salt has no effect, positive or negative, on the induction of skin tumors by UV light exposure. This is a TSCATS study (EPA Doc. No. 88-920007009; OTS0545384). See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/16090021.pdf
C.I. Fluorescent Brightener 260, disodium salt was not carcinogenic in this study.

Dominant Lethal

C.I. Fluorescent Brightener 28, disodium salt (CASRN 4193-55-9)
Twenty male NMRI mice were administered a single dose of C.I. Fluorescent Brightener 28, disodium salt at 5000 mg/kg-bw by gavage in a dominant lethal assay. Each night, one male was paired with three females throughout a week unless insemination was observed by inspection for a vaginal plug. The matings were repeated with three female mice/week over a period of 8 weeks. At gestational day 14, the uteri were examined to determine the number of implants and live and dead embryos. Methyl methanesulphonate (100 mg/kg-bw i.p.) was tested as the positive control and returned the expected results. No effects on fertility or uterine parameters were noted and there was no evidence for dominant lethal mutations following treatment of male mice with C.I. Fluorescent Brightener 28, disodium salt. See human health data at:
http://webnet.oecd.org/hpv/UI/Search.aspx
C.I. Fluorescent Brightener 28, disodium salt did not induce dominant lethality in mice in this assay.

C.I. Fluorescent Brightener 220, tetrasodium salt (CASRN 16470-24-9)
(1) Male NMRI mice were administered C.I. Fluorescent Brightener 220 as a single dose by gavage at 2500 or 5000 mg/kg-bw/day in a rodent dominant lethal test. A study description was not provided. There were no signs of toxicity in any group and no treatment-related effects on fertility or mutagenicity. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
C.I. Fluorescent Brightener 220, tetrasodium salt did not induce dominant lethality in this assay.

(2) Male NMRI mice were administered a single dose of C.I. Fluorescent Brightener 220, tetrasodium salt at 2500 or 5000 mg/kg-bw by gavage in a dominant lethal assay. Study details were not provided; however, there were no signs of toxicity in all treated groups, no effect on fertility and no effect on the number of implantation sites, resorption sites or live embryos

compared to controls. See human data at:
http://www.chem.unep.ch/irptc/sids/OECDSIDS/FLUORESCENT.pdf
C.I. Fluorescent Brightener 220, tetrasodium salt did not induce dominant lethality in this assay.

Conclusion: The acute oral toxicity of the stilbene fluorescent brighteners category members in rats, mice, rabbits, and dogs is low. The acute inhalation toxicity in rats is moderate (C.I. Fluorescent brightener) to high (C.I. Fluorescent brightener 28). The acute dermal toxicity in rats is low (C.I. Fluorescent brighteners 220, 235, and C.I. Fluorescent brightener) to moderate (C.I. Fluorescent brightener 263). In a combined oral chronic toxicity/carcinogenicity test, dietary exposure of rats to C.I. Fluorescent brightener 28 (free acid) for two years did not show effects considered to be treatment-related; the NOAELs were 543 and 779 mg/kg-bw/day (highest dose tested) for males and females, respectively. In a combined chronic toxicity/carcinogenicity test, dietary exposure of C.I. Fluorescent Brightener 28, disodium salt to rats for two years produced no adverse effects; the NOAEL was 500 mg/kg-bw/day (the highest dose tested). In a repeat dose study, administration of C.I. Fluorescent Brightener, via gavage, at 1000 mg/kg-bw/day for 28 days to Wistar rats resulted in hematology effects indicative of hemolytic anemia; the NOAEL was 200 mg/kg-bw/day. In a thirteen week study, repeat exposure to Fluorescent Brightener 263 by gavage to rats produced no adverse effects; the NOAEL was 300 mg/kg-bw/day (the highest dose tested). In a combined chronic toxicity/carcinogenicity test, dietary exposure of Fluorescent Brightener 220 to rats for two years produced no adverse effects; the NOAEL was 521/709 mg/kg-bw/day (the highest dose tested; males/females). In a 28 day study, repeat exposure to C. I. Fluorescent Brightener 260 by gavage to rats produced no adverse effects; the NOAEL was 1000 mg/kg-bw/day (the highest dose tested). In a two year study, dietary exposure of C. I. Fluorescent Brightener 260 to rats produced no adverse effects; the NOAEL was 524 and 791 mg/kg-bw/day (the highest dose tested) for males and females, respectively. In a two-generation reproductive toxicity study, repeated gavage exposure of albino rats to 1000 mg/kg-bw/day of C. I**.** Fluorescent Brightener resulted in increased relative and absolute kidney weights in parental females; the NOAEL (systemic toxicity; females) was 300 mg/kg-bw/day. The NOAEL (systemic toxicity; males) was 1000 mg/kg-bw/day (highest dose tested). No treatment-related effects on reproductive parameters, development, or growth of the F1 and F2 offspring were noted; the NOAEL (reproductive toxicity and growth and development) was 1000 mg/kg-bw/day (highest dose tested). In a 2-generation reproductive toxicity study, repeated gavage exposure of rats to C. I. Fluorescent Brightener 220 resulted in no adverse effect on reproductive performance and no adverse, test article-related changes in growth or development of offspring; the NOAEL for reproductive toxicity was 1000 mg/kg-bw/day. However, at 1000 mg/kg-bw/day there was an increase in absolute and relative kidney weights in P and F1 dams and F1 males. The NOAEL for systemic toxicity was 300 mg/kg-bw/day. In a prenatal developmental toxicity study, administration of C.I. Fluorescent Brightener by gavage to female Sprague-Dawley rats resulted in no adverse maternal or fetal effects; the NOAEL **(**maternal and developmental toxicity) was 1000 mg/kg-bw/day (highest dose tested). In a prenatal development study, administration of C. I. Fluorescent Brightener 220 via gavage to rabbits showed reduced discolored stools, early delivery, and abortion in the dams at 400 mg/kg-bw/day; the NOAEL for maternal toxicity and developmental toxicity was 100 mg/kg-bw/day. The stilbene fluorescent brighteners category members did not induce gene mutation or chromosomal aberrations. Excepting C.I Fluorescent

brightener 260, tested members of the Stilbene fluorescent brighteners category are not irritating to rabbit skin, Stilbene fluorescent brighteners category members C.I. Fluorescent Brightener 28, free acid, Fluorescent Brightener 220, and Fluorescent Brightener 263 are not irritating to rabbit eyes. Fluorescent Brightener 28, disodium salt and Fluorescent Brightener 260 are irritating to rabbit eyes. Fluorescent Brightener 28, disodium salt, Fluorescent Brightener 260, and 263 are not skin sensitizers in guinea pigs. The stilbene fluorescent brighteners category members are not considered carcinogenic. C.I. Fluorescent Brightener 28, disodium salt and C.I. Fluorescent Brightener 220 did not induce dominant lethal mutations in mice.

Table 3. Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Human Health Data

Endpoint	SPONSORED CHEMICAL C.I. Fluorescent Brightener 28, free acid (4404-43-7)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 28, disodium salt (4193-55-9)	SPONSORED CHEMICAL C.I. Fluorescent Brightener (13863-31-5)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 260, disodium salt (16090-02-1)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 220, tetrasodium salt (16470-24-9)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 235, tetrasodium salt (29637-52-3)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 263, tetrasodium salt (67786-25-8)
Acute Oral Toxicity LD_{50} (mg/kg-bw)	**> 15,000**	**> 15,000**	**> 5000**	**> 5000**	**> 15,000**	No Data > 2500 (RA)	**> 2500**
Acute Inhalation Toxicity LC_{50} (mg/L)	**> 1.82**	No Data > 1.82 (RA)	**> 2.9**	No Data > 2.9 (RA)	No Data > 2.9 (RA)	No Data > 2.9 (RA)	No Data > 2.9 (RA)
Acute Dermal Toxicity LD_{50} (mg/kg-bw)	No Data > 2000 (RA)	No Data > 2000 (RA)	**>2000**	**> 2000**	**> 2000**	No Data > 500 (RA)	**> 500**
Repeated-Dose Toxicity NOAEL/ LOAEL Oral (mg/kg-bw/day)	**NOAEL ~ 543/779** (highest dose tested;males/females)	**NOAEL ~ 500** (highest dose tested)	**NOAEL = 200 LOAEL = 1000**	**NOAEL = 524/791** (highest dose tested males/females)	**NOAEL = 521/709** (highest dose tested males/females)	No Data NOAEL =300 (RA)	**NOAEL = 300** (highest dose tested)

Table 3. Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Human Health Data

Endpoint	SPONSORED CHEMICAL C.I. Fluorescent Brightener 28, free acid (4404-43-7)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 28, disodium salt (4193-55-9)	SPONSORED CHEMICAL C.I. Fluorescent Brightener (13863-31-5)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 260, disodium salt (16090-02-1)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 220, tetrasodium salt (16470-24-9)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 235, tetrasodium salt (29637-52-3)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 263, tetrasodium salt (67786-25-8)
Reproductive Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day)							
Systemic Toxicity	No Data NOAEL = 300 LOAEL = 1000	No Data NOAEL = 300 LOAEL = 1000	**NOAEL=300 females** **LOAEL=1000 females** **NOAEL =1000 Males (highest dose tested)**	No Data NOAEL = 300 LOAEL = 1000	**NOAEL = 300** **LOAEL = 1000**	No Data NOAEL = 300 LOAEL = 1000	No Data NOAEL = 300 LOAEL = 1000
Reproductive Toxicity	NOAEL = 1000 (RA)	NOAEL = 1000 (RA)	**NOAEL=1000** (highest dose tested)	NOAEL = 1000 (RA)	**NOAEL = 1000** (highest dose tested)	NOAEL = 1000 (RA)	NOAEL = 1000 (RA)
Developmental Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day)							
Maternal Toxicity	No Data NOAEL = 1000	No Data NOAEL = 1000	**NOAEL = 1000** (highest dose tested)	No Data NOAEL = 1000	**NOAEL = 100** **LOAEL = 400**	No Data NOAEL = 100 LOAEL = 400	No Data NOAEL = 100 LOAEL = 400
Developmental Toxicity	NOAEL = 1000 (RA)	NOAEL = 1000 (RA)	**NOAEL = 1000** (highest dose tested)	NOAEL = 1000 (RA)	**NOAEL = 100** **LOAEL = 400**	NOAEL = 100 LOAEL = 400 (RA)	NOAEL = 100 LOAEL = 400 (RA)
Genetic Toxicity – Gene Mutation ***In vitro***	No Data Negative (RA)	**Negative**	**Negative**	**Negative**	**Negative**	No Data Negative (RA)	No Data [Negative] (RA)
Genetic Toxicity – Chromosomal Aberrations ***In vitro***	No Data Negative (RA)	No Data Negative (RA)	**Negative**	**Negative**	**Negative**	No Data Negative (RA)	No Data Negative (RA)
Genetic Toxicity – Chromosomal Aberrations ***In vivo***	No Data Negative (RA)	No Data Negative (RA)	**Negative**	**Negative**	**Negative**	No Data Negative (RA)	No Data Negative (RA)
Additional Information							
Skin Irritation	**Not irritating**	**Not irritating**	–	**Moderately irritating**	**Not irritating**	–	**Not irritating**
Eye Irritation	**Not irritating**	**Slightly irritating**	–	**Slightly irritating**	**Not irritating**	–	**Not irritating**
Sensitization	–	**Not sensitizing**	–	**Not sensitizing**	–	–	**Not sensitizing**
Carcinogenicity	–	**Negative**	–	**Negative**	–	–	**–**
Dominant Lethal	–	**Negative**	–	–	**Negative**	–	–

Measured data in bold; (RA) = Read Across; – indicates that endpoint was not evaluated for this substance

4. Hazard to the Environment

A summary of aquatic toxicity data submitted for SIDS endpoints is provided in Table 4. The table also indicates where data for tested category members are read-across (RA) to untested members of the category.

Acute Toxicity to Fish

C.I. Fluorescent Brightener 28, free acid (CASRN 4404-43-7)
(1) Fathead minnows (*Pimephales promelas*) were exposed to C.I. Fluorescent Brightener 28, free acid at five measured concentrations, the highest being 180 mg/L, under static conditions for 96 hours. A range-finding test and control were also conducted.
96-h LC_{50} > 180 mg/L

(2) Zebra fish (*Danio rerio*) were exposed to C.I. Fluorescent Brightener 28, free acid at unspecified concentrations under static conditions for 96 hours. A range-finding test and control was also conducted.
96-h LC_{50} = 5,382 mg/L

C.I. Fluorescent Brightener (CASRN 13863-31-5)
(1) Rainbow trout (*Onchorynchus mykiss*) were exposed to C.I. Fluorescent Brightener at unspecified concentrations under static conditions for 96 hours.
96-h LC_{50} = 108 mg/L

(2) Channel catfish (*Ictalurus punctatus*) were exposed to C.I. Fluorescent Brightener at unspecified concentrations under static conditions for 96 hours.
96-h LC_{50} = 86 mg/L

(3) Bluegill sunfish (*Lepomis macrochirus*) were exposed to C.I. Fluorescent Brightener at unspecified concentrations under static conditions for 96 hours.
96-h LC_{50} = 26 mg/L

C.I. Fluorescent Brightener 263, tetrasodium salt (CASRN 67786-25-8)
Zebra fish (*Danio rerio*) were exposed to C.I. Fluorescent Brightener 263, tetrasodium salt at nominal concentrations of 100, 1131, 2263, 4526, 10,000, 12,800 or 14,142 mg/L under static conditions for 96 hours.
96-h LC_{50} = 7,611 mg/L

C.I. Fluorescent Brightener 220, tetrasodium salt (CASRN 16470-24-9)
(1) Zebra fish (*Danio rerio*) were exposed to C.I. Fluorescent Brightener 220, tetrasodium salt at nominal concentrations of 100, 316 or 1000 mg/L under semi-static conditions for 14 days.
14-d NOEC > 859 mg/L

(2) Zebra fish (*Danio rerio*) were exposed to C.I. Fluorescent Brightener 220, tetrasodium salt at nominal concentrations of 562 or 1000 mg/L under static conditions for 96 hours. Measured

concentrations were 577 and 1051 mg/L, respectively. No mortality and no changes in behavior or appearance were observed.
96-h LC_{50} > 1000 mg/L

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
(1) Zebra fish (*Danio rerio*) were exposed to C.I. Fluorescent Brightener 260, disodium salt (also known as FWA-1) at nominal concentrations of 0, 100, 316 or 1000 mg/L under semi-static conditions for 14 days. Test concentrations were renewed on days 2, 4, 7, 9 and 11 and verified analytically. The mean measured concentrations in the 100 and 1000 mg/L test solutions were 61.8 and 215.5 mg/L, respectively. The estimated actual concentration in the 316 mg/L test solution was 126.4 mg/L. Mortality was 100% at 1000 mg/L with the fish exhibiting lethargic swimming behavior on 3 days.
14-d LC_{50} = 165 mg/L

(2) Zebra fish (*Danio rerio*) were exposed to C.I. Fluorescent Brightener 260, disodium salt (non-fluorescent Z-isomer) at nominal concentrations of 17.8, 32, 56, 100, 178 or 316 mg/L under static conditions for 96 hours. Measured concentrations were 17.4, 31.1, 55.4, 99.7, 178.5 and 319.4 mg/L.
96-h LC_{50} > 319 mg/L

(3) Zebra fish (*Danio rerio*) were exposed to C.I. Fluorescent Brightener 260, disodium salt (fluorescent E-isomer) at nominal concentrations of 17.8, 32, 56, 100, 178 or 316 mg/L under static conditions for 96 hours. Measured concentrations were 16.1, 32.1, 57.5, 102.2, 185.3 and 337.2 mg/L. Temperature, pH and dissolved oxygen were monitored. No mortality was observed at any exposure level.
96-h LC_{50} > 337 mg/L

Acute Toxicity to Aquatic Invertebrates

C.I. Fluorescent Brightener 28, free acid (CASRN 4404-43-7)
Water fleas (*Daphnia magna*) were exposed to C.I. Fluorescent Brightener 28, free acid at 1, 10, 50, 100, 300, 500, 900 or 1000 mg/L under static conditions for 24 hours. C.I. Fluorescent Brightener 28, free acid could not be dissolved, but rather, formed a fine dispersion.
24-h EC_0 > 1,000 mg/L

C.I. Fluorescent Brightener 28, disodium salt (CASRN 4193-55-9)
Water fleas (*Daphnia magna*) were exposed to C.I. Fluorescent Brightener, disodium salt at 100 mg/L in a limit test under static conditions for 48 hours.
48-h EC_{50} > 100 mg/L

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
Water fleas (*Ceriodaphnia dubia*) were exposed to C.I. Fluorescent Brightener 260, disodium salt at unspecified measured concentrations for under semi static conditions for 48 hours.
48-h EC_{50} = 6.85 mg/L

C.I. Fluorescent Brightener 220, tetrasodium salt (CASRN 16470-24-9)
(1) Water fleas (*Daphnia magna*) were exposed to C.I. Fluorescent Brightener 220, tetrasodium salt at a nominal concentration of 100 mg/L under static conditions for 48 hours. Effective concentrations were 91 and 134 mg/L after 0 and 48 hours, respectively.
48-h EC_{50} > 113 mg/L

(2) Water fleas (*Daphnia magna*) were exposed to C.I. Fluorescent Brightener 220, tetrasodium salt at assumed nominal concentrations of 0, 62.5, 125, 250, 500 or 1000 mg/L under static conditions for 24 hours.
24-h EC_{50} > 1000 mg/L

C.I. Fluorescent Brightener 263, tetrasodium salt (CASRN 67786-25-8)
Water fleas (*Daphnia magna*) were exposed to C.I. Fluorescent Brightener 263, tetrasodium salt at 100 mg/L in a limit test under static conditions for 48 hours.
48-h EC_{50} > 100 mg/L

C.I. Fluorescent Brightener (CASRN 13863-31-5)
Water fleas (*Ceriodaphnia dubia*) were exposed to C.I. Fluorescent Brightener 260, disodium salt at unspecified measured concentrations under semi static conditions for 48 hours.
48-h EC_{50} = 42.5 mg/L

Toxicity to Aquatic Plants

C.I. Fluorescent Brightener 28, disodium salt (CASRN 4193-55-9)
Green algae (*Pseudokirchnierella subcapitata*) were exposed to C.I. Fluorescent Brightener, disodium salt at 100 mg/L in a limit test under static conditions for 72 hours. A negative control was tested concurrently and the oxygen concentration, pH and temperature were controlled throughout the test. Algae growth was slightly limited at 100 mg/L: 18.4 % for biomass and 5.3% for growth.
72-h EC_{50} > 100 mg/L (biomass)

C.I. Fluorescent Brightener 220, tetrasodium salt (CASRN 16470-24-9)
Green algae (*Scenedesmus subspicatus*) were exposed to C.I. Fluorescent Brightener 220, tetrasodium salt at 500 – 1000 mg/L for 96 hours.
96-h EC_{50} > 1000 mg/L

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)
Green algae (*Scenedesmus subspicatus*) were exposed to C.I. Fluorescent Brightener 260, disodium salt at nominal concentrations of 3.125, 6.25, 12.5, 25, 50, 100 or 200 mg/L for 96 hours. Precipitation was observed. Measured concentrations were not provided. Inhibition of growth was determined from the area under the growth curves. Positive and negative controls were tested concurrently. The 96-hour E_bC_{50} was 41.1 mg/L, the 96-hour NOEC was 25 mg/L and the 96-hour LOEC was 50 mg/L.
72-h EC_{50} = 81 mg/L

Chronic Toxicity to Aquatic Invertebrates

C.I. Fluorescent Brightener 220, tetrasodium salt (CASRN 16470-24-9)

Water fleas (*Daphnia magna*) were exposed to C.I. Fluorescent Brightener 220, tetrasodium salt at nominal concentrations of 10 – 100 mg/L under semi-static conditions for 21 days. Measured concentrations were 64 – 80.5% of nominal in freshly prepared medium and 63 – 66.8% after 72 hours.

LOEC = 31.6 mg/L
NOEC = 10 mg/L

C.I. Fluorescent Brightener 260, disodium salt (CASRN 16090-02-1)

Water fleas (*Daphnia magna*) were exposed to C.I. Fluorescent Brightener 260, disodium salt at nominal concentrations of 0, 1.0, 3.2, 10, 31.6 or 100 mg/L under semi-static conditions for 21 days. Test concentrations were verified analytically and nominal concentrations were corrected to effective concentrations using a mean recovery of 75% (the mean percentage recovery was 72.5 and 78.8% for the 1 and 100 mg/L concentrations, respectively).

LOEC = 2.4 mg/L
NOEC = 0.75 mg/L

Summary:

The 96-h LC_{50} of C.I. fluorescent brightener 28, free acid (CASRN 4404-43-7) for fish ranges from > 180 to 5,382 mg/L. The 96-h LC_{50} of C.I. fluorescent brightener (CASRN 13863-31-5) ranges from 26 to 108 mg/L. The 96-h LC_{50} of C.I. fluorescent brightener 263, tetrasodium salt (CASRN 67786-25-8) for fish is 7,611 mg/L. The 96-hLC_{50} of C.I. fluorescent brightener 220, tetrasodium salt (CASRN 16470-24-9) for fish ranges from 859 to 1,000 mg/L. The 96-h LC_{50} of C.I. fluorescent brightener 260, disodium salt (CASRN 16090-02-1) for fish ranges from 319 to 337 mg/L.

The 24-h EC_{50} of C.I. fluorescent brightener 28, free acid (CASRN 4404-43-7) for aquatic invertebrates is > 1,000 mg/L. The 48-h EC_{50} of C.I. fluorescent brightener 28, disodium salt (CASRN 4193-55-9) for aquatic invertebrates is > 100 mg/L. The 48-h EC_{50} of C.I. fluorescent brightener 260, disodium salt (CASRN 16090-02-1) for aquatic invertebrates is 6.85 mg/L. The 48-h EC_{50} of C.I. fluorescent brightener 220, tetrasodium salt (CASRN 16470-24-9) for aquatic invertebrates ranges from >113 mg/L to > 1,000 mg/L. The 48-hour EC_{50} of C.I. fluorescent brightener (CASRN 13863-31-5) for aquatic invertebrates is 42.5 mg/L.

The 72-h EC_{50} of C.I. fluorescent brightener 28, disodium salt (CASRN 4193-55-9) for aquatic plants is > 100 mg/L. The 96-h EC_{50} of C.I. fluorescent brightener 220, tetrasodium salt (CASRN 16470-24-9) for aquatic plants is > 1,000 mg/L. The 72-h EC_{50} of C.I. fluorescent brightener 260, disodium salt (CASRN 16090-02-1) for aquatic plants is 81 mg/L.

The 21-d LOEC and NOEC of C.I. fluorescent brightener 220, tetrasodium salt (CASRN 16470-24-9) for aquatic invertebrates is 31.6 mg/L and 10 mg/L respectively. The 21-d LOEC and NOEC of C.I. fluorescent brightener 260, disodium salt (CASRN 16090-02-1) for aquatic invertebrates is 2.4 mg/L and 0.75 mg/L, respectively.

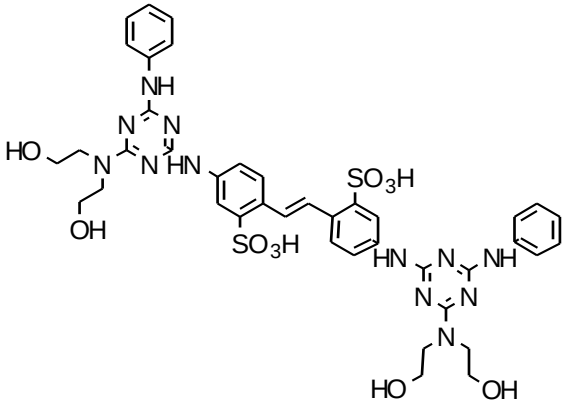
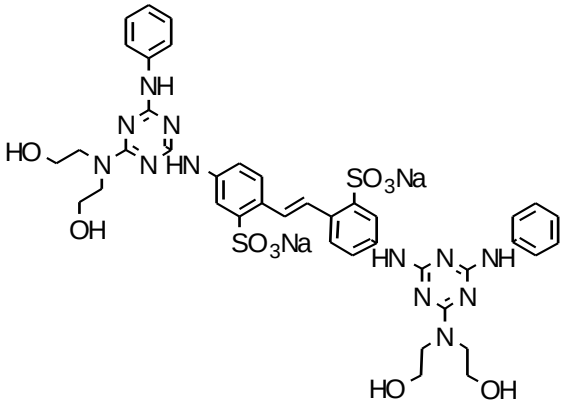
Conclusion: The 96-h LC_{50} of the stilbene fluorescent brightener chemicals for fish ranges from 26 to 7,611mg/L. The 48-h EC_{50} of the stilbene fluorescent brightener chemicals for aquatic invertebrates ranges from 6.85 to > 113 mg/L. The 72-h EC_{50} of the stilbene fluorescent brightener chemicals for aquatic plants ranges from 81 to > 100 mg/L. The 21-d LOEC and NOEC of the stilbene fluorescent brighteners chemicals for aquatic invertebrates ranges from 2.4 to 31.6 mg/L.

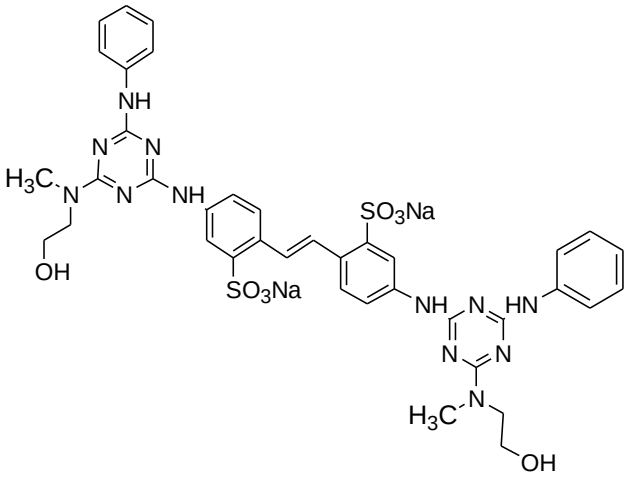
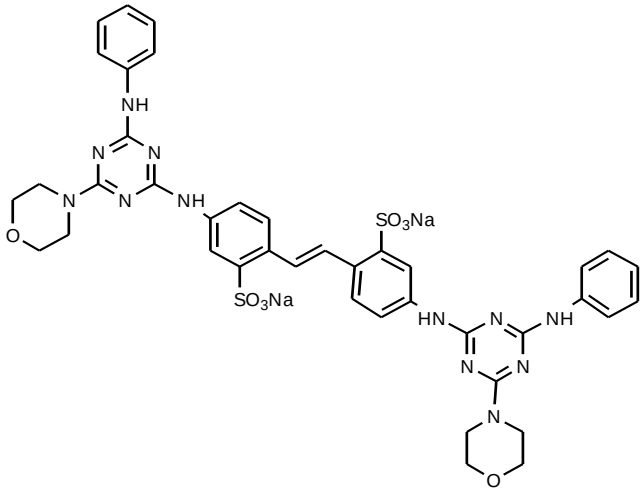
Table 4. Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Aquatic Toxicity Data

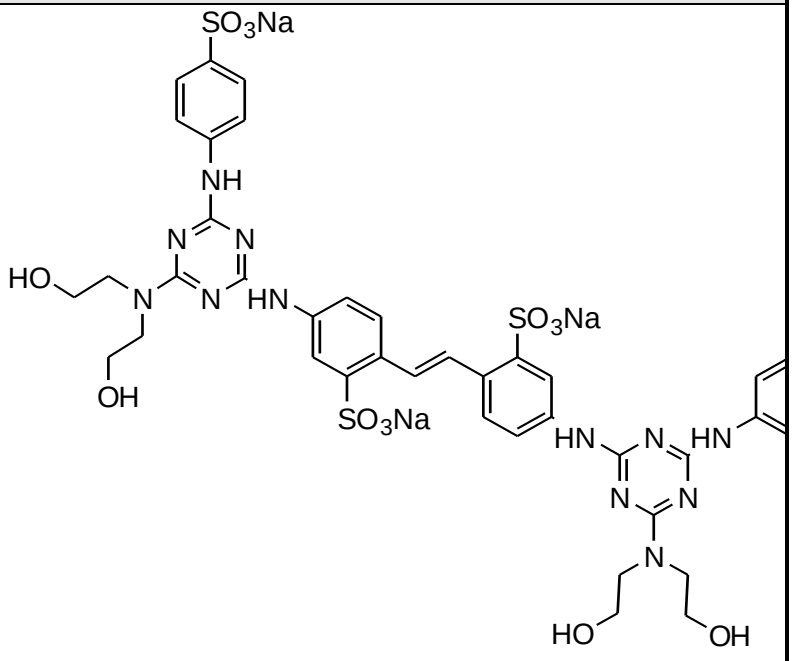
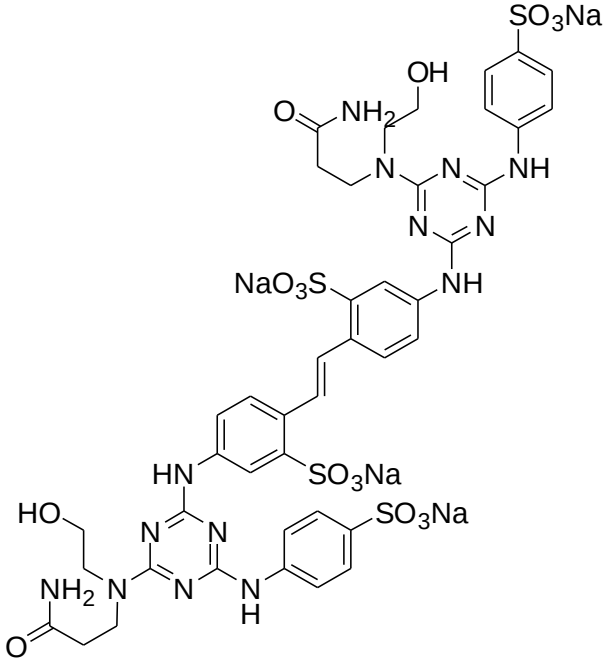
Endpoints	SPONSORED CHEMICAL C.I. Fluorescent Brightener 28, free acid (4404-43-7)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 28, disodium salt (4193-55-9)	SPONSORED CHEMICAL C.I. Fluorescent Brightener (13863-31-5)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 260, disodium salt (16090-02-1)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 220, tetrasodium salt (16470-24-9)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 235, tetrasodium salt (29637-52-3)	SPONSORED CHEMICAL C.I. Fluorescent Brightener 263, tetrasodium salt (67786-25-8)
Fish **96-h LC_{50} (mg/L)**	**> 180**	No Data > 180 (RA)	**26**	**> 337**	**> 1000**	No Data > 1000 (RA)	**7611**
Aquatic Invertebrates **48-h EC_{50} (mg/L)**	**> 1000 (24-h)**	**> 100**	**42.5**	**6.85**	**> 113**	No Data > 100 (RA)	**> 100**
Aquatic Plants **72-h EC_{50} (mg/L)**	No Data 81 (RA)	**> 100**	No Data 81 (RA)	**81**	**> 1000 (96-h)**	No Data > 1000 (RA)	No Data > 1000 (RA)
Chronic Toxicity to Invertebrates **21-d EC_{50} (mg/L)**	No Data LOEC 2.4 – 31.6 (RA)	No Data LOEC 2.4 – 31.6 (RA)	No Data LOEC 2.4 – 31.6 (RA)	**LOEC = 2.4**	**LOEC = 31.6**	No Data LOEC 2.4 – 31.6 (RA)	No Data LOEC 2.4 – 31.6 (RA)

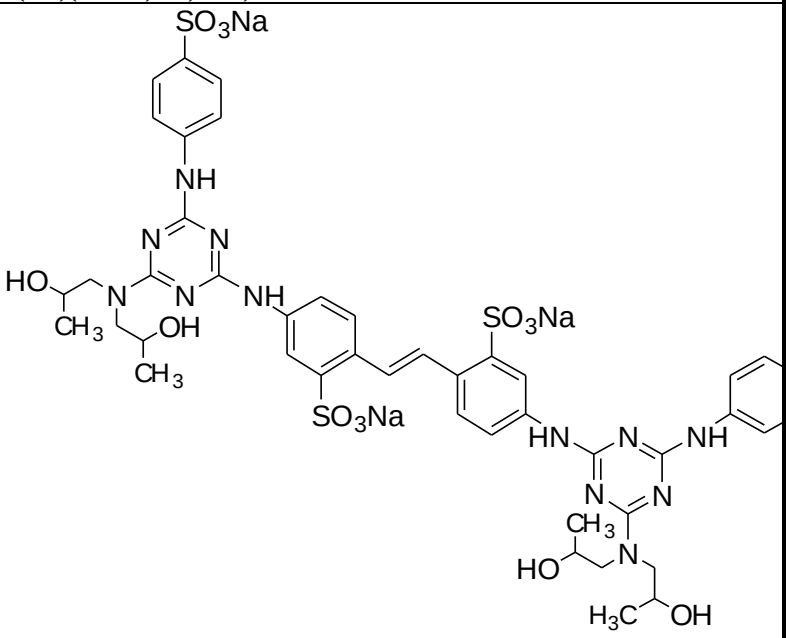
(m) = measured data (i.e., derived from testing); (RA) = Read Across

APPENDIX

Sponsored Chemicals		
Chemical Name	**CASRN**	**Structure**
Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-	4404-43-7	 SMILES: OCCN(C1=NC(NC6=CC=CC=C6)=NC(NC2=CC=C(C=CC3=C(S(=O)(O)=O)C=C(NC4=NC(NC5=CC=CC=C5)=NC(N(CCO)CCO)=N4)C=C3)C(S(=O)(O)=O)=C2)=N1)CCO
Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2)	4193-55-9	 SMILES: OCCN(C1=NC(NC6=CC=CC=C6)=NC(NC2=CC=C(C=CC3=C(S(=O)(O[Na])=O)C=C(NC4=NC(NC5=CC=CC=C5)=NC(N(CCO)CCO)=N4)C=C3)C(S(=O)(O[Na])=O)=C2)=N1)CCO

Sponsored Chemicals		
Chemical Name	**CASRN**	**Structure**
Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[(2-hydroxyethyl)methylamino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2)	13863-31-5	 SMILES: CN(C1=NC(NC3=CC(S(=O)(O[Na])=O)=C(C=CC4=CC=C(NC5=NC(N(CCO)C)=NC(NC6=CC=CC=C6)=N5)C=C4S(=O)(O[Na])=O)C=C3)=NC(NC2=CC=CC=C2)=N1)CCO
Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-(4-morpholinyl)-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, sodium salt (1:2)	16090-02-1	 SMILES: O=S(C1=C(C=CC5=CC=C(NC6=NC(N7CCOCC7)=NC(NC8=CC=CC=C8)=N6)C=C5S(=O)(O[Na])=O)C=CC(NC2=NC(NC4=CC=CC=C4)=NC(N3CCOCC3)=N2)=C1)(O[Na])=O

Sponsored Chemicals		
Chemical Name	**CASRN**	**Structure**
Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, sodium salt (1:4)	16470-24-9	 SMILES: OCCN(C1=NC(NC6=CC=C(S(=O)(O[Na])=O)C=C6)=NC(NC2=CC=C(C=CC3=C(S(=O)(O[Na])=O)C=C(NC4=NC(NC5=CC=C(S(=O)(O[Na])=O)C=C5)=NC(N(CCO)CCO)=N4)C=C3)C(S(=O)(O[Na])=O)=C2)=N1)CCO
Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[(3-amino-3-oxopropyl)(2-hydroxyethyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, sodium salt (1:4)]	29637-52-3	 SMILES: OCCN(CCC(N)=O)C1=NC(NC6=CC=C(S(=O)(O[Na])=O)C=C6)=NC(NC2=CC=C(C=CC3=C(S(=O)(O[Na])=O)C=C(NC4=NC(NC5=CC

Sponsored Chemicals		
Chemical Name	**CASRN**	**Structure**
		=C(S(=O)(O[Na])=O)C=C5)=NC(N(CCC(N)=O)CCO)=N4)C=C3)C(S(=O)(O[Na])=O)=C2)=N1
Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxypropyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, sodium salt (1:4)]	67786-25-8	 SMILES: OC(C)CN(C1=NC(NC6=CC=C(S(=O)(O[Na])=O)C=C6)=NC(NC2=CC=C(C=CC3=C(S(=O)(O[Na])=O)C=C(NC4=NC(NC5=CC=C(S(=O)(O[Na])=O)C=C5)=NC(N(CC(C)O)CC(C)O)=N4)C=C3)C(S(=O)(O[Na])=O)=C2)=N1)CC(C)O